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(54) Title: METHOD OF ANESTHESIA (57) Abstract A <u>method of producing local anesthesia in a mammal experiencing pain in an epithelial tissue region</u> is described. The method includes topically administering to the region, in a suitable pharmaceutical vehicle, an effective dose of a <u>long-acting sodium channel blocking compound</u> . <div style="text-align: center; margin-top: 100px;"><i>Torini + Grant</i></div>		

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METHOD OF ANESTHESIA

Cross Reference to Related Applications

- 5 This application claims the benefit of U.S. Provisional Application No. 60/040,903, filed April 2, 1997 and U.S. Provisional Application No. 60/076,317, filed February 27, 1998.

Field of the Invention

- 10 The present invention relates to a method for producing local anesthesia by topical administration of sodium channel blocking compounds, including tetrodotoxin and saxitoxin.

Background of the Invention

- 15 Pain is a well known phenomenon as an indicator of actual or potential injury or tissue damage due to inflammation, ischemia, mechanical or other irritation. Treatment of pain includes the use of local anesthetics, which block neuronal transmission and affect sensation as well as pain, and analgesics, which relieve pain and additionally may interfere with the activity of chemical mediators of
20 inflammation.

Loss or damage of epithelial tissue is usually associated with moderate to severe pain and can result from a number of causes, for example, burns, corneal abrasions, other abnormalities of mucosal tissues, and surgical procedures involving epithelial and other tissues.

- 25 An example of pain associated with a surgical procedure is surgical correction of myopia by excimer laser photorefractive keratectomy. Following photorefractive keratectomy (PRK), patients generally experience moderate to severe eye pain in the first 24 to 48 hours. Current pain management with bandage contact lens, non-steroidal anti-inflammatory agents, and oral analgesics mitigates, but does not
30 eliminate, the discomfort in most patients (Cherry, Tutton). Topical anesthetics have been used to reduce pain, but due to their short duration of action, frequent administration is required. For example, benoxinate, cocaine, tetracaine, and proparacaine are commonly prescribed topical anesthetics for management of eye pain. These topical anesthetics only provide pain relief for short periods, on the order

of 15 to 30 minutes. Given frequently, these agents can be toxic to the corneal epithelium and inhibit re-epithelialization (Rosenwasser).

Thus, there is a need in the art for methods of producing long-lasting, local anesthesia without inhibiting re-epithelialization or healing of other tissues.

5

Summary of the Invention

The methods and compositions of the present invention solve, inter alia, the long-recognized need in the art for methods of producing local anesthesia of long duration. In the particular embodiment of producing long-lasting local anesthesia of the corneal surface of an eye, the inventors have addressed a problem of great clinical significance, showing for the first time that sodium channel blocking compounds, such as tetrodotoxin and saxitoxin, can produce ocular surface anesthesia of long duration without impairing re-epithelialization. Moreover, the inventors have shown that the effective doses of these sodium channel blocking compounds have a wide margin of safety and that systemic absorption of tetrodotoxin topically administered to abraded corneas is low.

The methods and compositions of the invention can be used for any condition involving ocular surface pain, including local anesthesia following ocular surgery, including PRK, and following injury to the eye. The methods provide significant advantages, including providing at least 3 hours, preferably at least 4 hours, more preferably at least 6 hours, and most preferably, at least 8 hours of local anesthesia without affecting re-epithelialization of the corneal surface (e.g. wound healing).

The present invention includes methods of producing long-lasting local anesthesia, comprising administering a pharmaceutically acceptable composition of a long-acting sodium channel blocking compound, wherein said compound binds to the extracellular mouth of the sodium channel, occluding the channel by a mechanism separate from that of local anesthetics, such as proparacaine. Preferably, such methods achieve local anesthesia of long duration, lasting at least 3 hours (3 to 10 hours), preferably at least 4 hours (4-10 hours), and most preferably at least 6 to 10 hours. Preferred compounds include toxins or analogs thereof that specifically bind to a site formed in part by an extracellular region of the alpha subunit of a sodium channel. Most preferred compounds comprise the class of toxins and analogs that specifically bind to a site formed by the SS1 and SS2 extracellular regions of the

alpha subunit of a sodium channel, wherein such compounds include tetrodotoxin, saxitoxin and analogs thereof. Surprisingly, these long-acting sodium channel blocking compounds, which are well known, potent neurotoxins, provide long-lasting local anesthesia without inhibiting reepithelialization.

5 Accordingly, it is an object of the invention to provide a method of producing local anesthesia in patients experiencing pain associated with damage to epithelial tissue.

 It is another object of the invention to provide a method of producing local anesthesia for long-acting pain control.

10 It is another object of the invention to provide a method of producing local anesthesia in epithelial tissues having damage associated with corneal abrasions, as in, for example, ophthalmic surgery, such as post-operative photorefractive keratectomy, other forms of corneal refractive surgery, including excimer laser PRK and LASIK, without impairing healing of the epithelial tissue. Other applications include, but are
15 not limited to, any condition where ocular surface anesthesia of long duration is desired, including after surgery or injury

 In one aspect, the invention includes a method of producing local anesthesia in a subject experiencing pain in an epithelial tissue region. The method includes
20 topically administering to the region, in a suitable pharmaceutical vehicle, an effective dose of tetrodotoxin or saxitoxin.

 In one embodiment, the effective dose of tetrodotoxin or saxitoxin is administered from a formulation containing tetrodotoxin or saxitoxin at a concentration of between 0.001 mM and 10 mM.

 In another embodiment, tetrodotoxin or saxitoxin is administered to a de-
25 epithelialized corneal tissue region. For example, tetrodotoxin is administered to the eye following excimer laser photorefractive keratectomy by instillation of drops. In this application, tetrodotoxin is typically formulated in a vehicle having a pH of between 4-8, more preferably between about 5-7.5.

 In one embodiment, tetrodotoxin or saxitoxin is administered topically every
30 6-8 hours for between about 24-72 hours.

 The method of the invention, in another embodiment, is for producing local anesthesia by topical administration of tetrodotoxin to an epithelial tissue region in the upper or lower gastrointestinal tract. In other embodiments, the epithelial tissue

region is associated with genital lesions in the genital area, with epithelial tissue region is in the esophagus, or with facial epithelial tissue.

In another aspect, the invention includes a method of producing local anesthesia in the eye of a mammalian subject, by topically administering to the
5 corneal surface of the eye of the subject, in a pharmaceutically suitable vehicle, a pharmaceutically effective dose of tetrodotoxin, saxitoxin or other long-acting sodium channel blocking compound. Such methods find application whenever ocular surface anesthesia is desired.

In one embodiment of this aspect, the corneal surface is partially or
10 completely de-epithelialized.

In a third aspect, the invention includes a method of reducing pain following corneal surgery, including but not limited to excimer laser photorefractive keratectomy, by topically administering to the corneal surface of the eye of the
15 subject, in a pharmaceutically suitable vehicle, a pharmaceutically effective dose of tetrodotoxin or saxitoxin.

In one embodiment of the third aspect, the method also includes the step of instilling drops of a non-steroidal anti-inflammatory compound in the eye of the subject. In other embodiments, the method further includes applying a bandage contact lens to the eye of the subject, or administering an antibiotic, steroid, or non-
20 steroidal anti-inflammatory drug to the subject. Methods of administering combination drops to the eye are also contemplated, wherein such drops contain a composition comprising a long-acting sodium channel blocking compound and an antibiotic, steroid, or nonsteroidal anti-inflammatory drug in an ophthalmically acceptable vehicle.

In yet another embodiment, the invention includes methods of producing local
25 anesthesia in an eye of a mammal, comprising (a) topically administering to the corneal surface of the eye of said mammal, in a pharmaceutically suitable vehicle, 0.05%-0.5% proparacaine and (b) topically administering to the corneal surface of the eye of said mammal, in a pharmaceutically suitable vehicle, an ophthalmically
30 effective dose of a long-acting sodium channel blocking compound, wherein step (b) follows step (a). In a preferred embodiment, a 0.05% proparacaine concentration is administered.

Also provided are compositions of a long-acting sodium channel blocking compound in an ophthalmically acceptable pH range of between 4 –8, wherein the concentration of said compound is not greater than 1 mM (0.01 mM to 1 mM), preferably not greater than 0.5 mM (0.01mM to 0.5 mM), more preferably not greater than 0.2 mM (0.01 mM to 0.2 mM), and most preferably not greater than 0.1 mM (0.01 mM to 0.1 mM).

These and other objects and features of the invention will be more fully appreciated when the following detailed description of the invention is read in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A is a plot showing corneal blink response in rabbit eyes as a function of time after administration to the cornea with intact epithelium of 0.1 mM (open circles), 1 mM (open diamonds) and 10 mM (open squares) tetrodotoxin;

Fig. 1B is a plot showing corneal blink response in fellow rabbit eyes for Fig. 1A following topical administration of the vehicle only control;

Fig. 2A is a plot showing corneal blink response in rabbit eyes with intact corneal epithelium as a function of time following topical administration of 1 mM tetrodotoxin, 10 mM tetrodotoxin or a comparative anesthetic, 0.5% proparacaine;

Fig. 2B is a plot showing corneal blink response in fellow rabbit eyes for Fig. 2A following administration of the vehicle only control;

Figs. 3A-3B are plots showing corneal blink response in rabbit eyes following central corneal epithelial debridement and administration of 0.01 mM (open circles), 0.1 mM (open diamonds) or 1.0 mM tetrodotoxin (open squares) (Fig. 3A) and of the fellow eyes treated with a vehicle only control (Fig. 3B);

Fig. 4 is a plot showing corneal blink response as a function of time in rabbit eyes with central corneal epithelial debridement treated with doses of 1.0 mM tetrodotoxin administered every 6 hours for 24 hours; and

Fig. 5 is a plot showing the size of a corneal epithelial defect, in mm², as a function of time for untreated rabbit eyes (open squares) and for rabbit eyes treated with topical 1 mM tetrodotoxin every 8 hours (open diamonds).

Fig. 6 is a chart showing administration of TTX to produce corneal anesthesia of rabbit eyes post-excimer laser keratectomy as measured by a blink response test.

Corneal sensation was tested at 3, 6, 9, 12, 15, 18, 21, 24, 30, 32 and 40 hours after excimer laser keratectomy. One 40 microliter drop of 1 mM TTX or vehicle (control) was administered at 0, 6, 12, 18 and 24 hours, as shown by the arrows. The results are graphed as the blink response (mean +/- SEM; N=6).

5 **Fig. 7** is a chart showing the effect of topical administration of TTX on corneal wound healing following excimer laser keratectomy of rabbit eyes. Wound healing was assessed by measuring the size of the epithelial defect remaining at 24, 40, 49, 63, 68 and 72 hours after excimer laser keratectomy. The results are given as the area of the epithelial defect remaining in either the eyes treated with 1 mM TTX
10 or the fellow eyes treated with the vehicle without TTX (control) (mean +/-SD; N=6).

Fig. 8 is a chart showing corneal anesthesia in abraded rabbit eyes following topical application of 20 microliters of 0.1 mM TTX. Corneal sensation was tested for TTX-treated and vehicle treated (control) eyes at 4 and 6 hours after a single topical administration of TTX or the control. The chart shows that a single 20 ul dose
15 of 0.1 mM TTX, when applied to abraded rabbit corneas, resulted in 6 hours of local anesthesia as measured by the blink response.

Fig. 9 is a chart showing corneal anesthesia in abraded rabbit eyes following topical application of 20 microliters of 0.2 mM TTX. Corneal sensation was tested for TTX-treated and vehicle treated (control) eyes at 4, 6 and 8 hours after a single
20 topical administration of TTX or the control. The chart shows that a single 20 ul dose of 0.2 mM TTX, when applied to abraded rabbit corneas, results in 8 hours of local anesthesia as measured by the blink response.

Fig. 10 is a chart showing corneal sensitivity after topical administration of a single dose of either 1 mM STX or vehicle (control) to abraded rabbit corneas as
25 measured by blink response at 2, 4 and 6 hours after administration.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

30 The following terms, as referred to herein, have the following meanings, unless otherwise indicated.

 “Long-acting sodium channel blocking compound” refers to a compound, e.g. a toxin or analog that, when administered to a mammal in an effective concentration.

causes local anesthesia lasting at least 3 to 10 hours, and specifically binds to the extracellular mouth of the sodium channel, occluding the channel by a mechanism separate from that of local anesthetics, such as lidocaine, proparacaine. See J.F. Butterworth and G.R. Strichartz, *Anesthes.* 72:711-734 (1990). Long-acting sodium channel blocking compounds, when administered in a single dose, may effect local anesthesia of long duration, lasting at least 3 hours (3 to 10 hours), preferably at least 4 hours (4-10 hours), and most preferably at least 6 to 10 hours. Such long-acting sodium channel blocking compounds include compounds that specifically bind to a site formed in part by an extracellular region of the alpha subunit of a sodium channel. See Goodman & Gilman's, *The Pharmacological Basis of Therapeutics*, Ninth Edition 340-341 (1996); H. Terlau, et al., *Fed. Europ. Biochem. Soc.* 293(1-2): 93-96 (1991); *Encyclopedia of Molecular Biology*, pages 1127-1131 (ed. J. Kendrew 1994). . Examples of long-acting sodium channel blocking compounds that bind to an extracellular site formed by the SS1 and SS2 segments of the alpha subunit include but are not limited to tetrodotoxin, saxitoxin, chiriquitoxin, GTTX (from *G. tamarensis*), gonyautoxins (GTX-I-V, GTX-I, GTX-II, GTX-III), neosaxitoxin, and derivatives and analogs thereof. D.J. Bower, et al., *Clinical Toxicology*, 18(7):813-863 (1981). Examples of long-acting sodium channel blocking compounds that bind to an extracellular site formed by the SS3 and SS4 segments of the alpha subunit, include but are not limited to alpha-scorpion toxin and sea anemone toxin. See Rogers, J.C. et al., *J. Biol. Chem.* 271(27):15950-15962 (1996).

"Saxitoxin" or "STX" refers to a compound comprising a tetrahydropurine moiety composed of two guanidine units fused together in a stable azaketal linkage, having a molecular formula $C_{10}H_{27}N_7O_4 \cdot 2-HCl$, (mol. wt. 299.30) and to derivatives thereof, including but not limited to hydroxysaxitoxins and neosaxitoxin. Bower et al., *Nonprotein Neurotoxins*, *Clin. Toxicol.* 18(7):813-863 (1981).

"Tetrodotoxin" or "TTX" refers to the amino perhydroquinazoline compound having the molecular formula $C_{11}H_{17}N_3O_8$ and to derivatives thereof, including but not limited to anhydrotetrodotoxin, tetrodaminotoxin, methoxytetrodotoxin, ethoxytetrodotoxin, deoxytetrodotoxin and tetrodonic acid (Kao). Examples of TTX analogs include novel TTX analogs isolated from other organisms, as well as those that are partially or totally chemically synthesized. See e.g., Yotsu, M. et al. *Agric.*

Biol. Chem., 53(3):893-895 (1989). Such analogs bind to the same site on the alpha subunit of sodium channels as does TTX.

"Anesthesia" refers to the loss of sensation, and, as used herein encompasses analgesia, the reduction in perceived pain without necessarily loss of sensation.

5 "Topical administration or topically administering" refers to application to a tissue of a mammal, including but not limited to application to epithelial tissue which has been damaged, lost or de-epithelialized.

"Epithelial tissue region" refers to an area of epithelial tissue in a mammal, where the epithelial layer is intact, damaged, or partially or completely absent.

10 "Pharmaceutically acceptable dose" refers to administration of an amount of a long-acting sodium channel blocking compound effective to achieve a local anesthetic effect for a clinically useful period of time.

"Ophthalmically acceptable dose" refers to administration of an amount of a long-acting sodium channel blocking compound effective to achieve a local
15 anesthetic effect in an eye for a clinically useful period of time.

II. Administration of Long-Acting Sodium Channel Blocking Compounds

The invention is directed to a method of providing local anesthesia to a mammal experiencing pain in a tissue, preferably an epithelial tissue region. The
20 method includes topically administering to the region, an effective dose of a long-acting sodium channel blocking compound in a suitable vehicle, including an ophthalmically suitable vehicle.

In general embodiments of the invention, described in more detail below, the method provides local anesthesia to a patient having pain in an epithelial tissue region
25 associated with damage or loss of epithelial tissue as a result of, for example, plastic surgery, canker sores, burns, sore throats, genital lesions, upper or lower gastrointestinal bronchoscopy or endoscopy, intubation, dermatologic abrasions or chemical skin peels.

In one preferred embodiment, the method is for producing local anesthesia in
30 de-epithelialized corneal tissue in the eye of a patient after injury to the eye, for example, photorefractive keratectomy, by topically administering to the eye an effective dose of a long-acting sodium channel blocking compound in a suitable vehicle.

In experiments performed in support of the present invention, the use of tetrodotoxin as a topical anesthetic was demonstrated by administration to corneal epithelial tissue in rabbit eyes. Tetrodotoxin was administered to healthy rabbit eyes and to de-epithelialized rabbit eyes to determine the extent and duration of local anesthesia provided by tetrodotoxin and to evaluate its toxicity, as will be described below.

A. Administration in Healthy Rabbit Eyes

1. Administration of 0.1 mM, 1 mM and 10 mM tetrodotoxin

The extent and duration of local anesthesia provided by tetrodotoxin was determined by administration of the anesthetic at concentrations of 0.1 mM, 1 mM and 10 mM to healthy rabbit eyes. As described in Example 1, tetrodotoxin was administered to the right eye of 18 rabbits by instillation of 40 μ l of the drug in a sodium citrate vehicle. The fellow, left eye of each rabbit received 40 μ l of the sodium citrate vehicle as a control.

The anesthetic effect was determined by the corneal blink test, as described in Example 1. In this test, the cornea of each rabbit was mechanically stimulated and the rabbit's response was scored on a scale of 1-3, where a score of 1 was assigned for no blink, a score of 2 was assigned for a partial blink without full lid closure and a score of 3 was assigned for a full blink. Each eye was scored prior to administration of the drug and at intervals after administration for 8 hours.

Fig. 1A shows the results of administration of tetrodotoxin, where the response score is plotted as a function of time after administration of 0.1 mM (open circles), 1 mM (open diamonds) and 10 mM (open squares) tetrodotoxin. At a concentration of 0.1 mM tetrodotoxin, no local anesthesia was produced in the rabbits, as evidenced by the response scores of 2.8-3.0.

The 1 mM tetrodotoxin formulation produced local anesthesia in all of the animals, as evidenced by the one minute score of 1.17. The local anesthesia effect was relatively short-lived, as evidenced by the 1 hour score of 1.5 and by the 3 hour score of 2.83.

The 10 mM tetrodotoxin formulation produced longer-lasting local anesthesia. At one minute after administration, all rabbit corneas were locally anesthetic with a mean anesthesia score of 1.0 (SD=0). At 4 hours, local anesthesia was still present

with a mean score of 1.17 (SD=0.41), and by 8 hours, the mean score of 2.0 indicated residual local anesthesia in most animals. As late as 8 hours, 5 of 6 rabbits showed some residual local anesthesia.

Fig. 1B shows the corneal response scores following administration of the placebo control vehicle. As seen, corneal blink response remained at a score of about 3, indicating that none of the eyes treated with the control vehicle had diminished corneal sensitivity.

2. Comparative Test

In a separate experiment (Example 1B), the anesthetic duration provided by tetrodotoxin (10 mM and 1 mM) was compared to that provided by 0.5% proparacaine, a common topical ocular anesthetic (Rosenwasser). Following the procedure set forth in Example 1, the right eye of each of the 18 test rabbits received 40 µl of the test drug with the fellow, left eye of each rabbit receiving a placebo control. The results are shown in Figs. 2A and 2B.

As seen in Fig. 2A, the 10 mM tetrodotoxin dose (open squares) produced significantly longer local anesthesia than proparacaine (open circles). While proparacaine produced local anesthesia in 6 of 6 rabbits at 1 minute (Table 2), by 1 hour the mean blink response score had increased to 2.50 (SD+0.84), and at 3 hours, all eyes receiving proparacaine had normal sensation. In contrast, as late as 5 hours, 4 of 6 rabbits receiving tetrodotoxin showed some residual local anesthesia with a mean score of 1.83, which was significantly different ($p=0.0325$, Wilcoxon test) from the mean score of 3.00 obtained with proparacaine at 5 hours.

3. Slit Lamp Examination, Pachometry, Toxicity

To assess whether administration of tetrodotoxin causes clinical alterations in the cornea, the eyes of animals treated with 10 mM and 1 mM tetrodotoxin were examined at 12 and 24 hours after drug administration by slit lamp biomicroscopy with a portable Kowa slit lamp, with and without fluorescein stain from impregnated strips moistened with balance salt solution. There was no apparent ocular irritation or epithelial toxicity after administration, nor was there any obvious discomfort, as evidenced by prolonged eye closure or repetitive blinking.

To evaluate whether endothelial function was significantly affected by administration of tetrodotoxin, pachometry (Humphrey Pachometer) readings prior to and 24 hours after tetrodotoxin administration were performed on animals treated with 10 mM and 1 mM tetrodotoxin. Pachometry readings on rabbit eyes receiving the highest doses of tetrodotoxin showed no evidence of corneal thickening during the 24 hour observation period, as shown in Table 3 in Example 1B.

With respect to systemic toxicity, each test rabbit was observed carefully for any signs of systemic toxicity during the 24 hour test period. No rabbit had any alterations of feeding, movement, respiration, or alertness during this period that might suggest a toxic effect of tetrodotoxin. No rabbit died or was noted to have abnormalities in behavior.

Collectively, and in summary, these experiments demonstrate that tetrodotoxin, administered topically by instillation of drops into the eye, provides an anesthetic effect. The dosage administered from the 10 mM tetrodotoxin formulation achieved corneal anesthesia with a rapid onset and anesthetic duration for nearly 4 hours, with some anesthetic effect, as evidenced by reduced corneal sensation, provided for at least 8 hours, a significant improvement over that provided by proparacaine. The tests also showed a dose response effect, with lower doses producing either shorter or no anesthetic effect. Importantly, there was no apparent signs of ocular irritation, corneal thickening or systemic toxicity after administration of tetrodotoxin.

B. Administration in De-Epithelialized Rabbit Eyes

In other experiments performed in support of the invention, tetrodotoxin was administered to de-epithelialized rabbit corneas. As set forth in Example 2A, a central epithelial corneal abrasion was created in each eye and tetrodotoxin, at a concentration of 0.1 mM, 1 mM or 10 mM, was administered into the inferior conjunctival cul-de-sac of one eye. A control vehicle was administered into the fellow eye.

Corneal sensation was tested, as described in Example 2A, and the rabbit's response was scored as described above on a scale of 1-3, with a score of 3 indicating full responsiveness and a score of 1 indicating full local anesthesia. Corneal sensation

was tested prior to administration of tetrodotoxin and after administration at 2, 4, 6 and 8 hours (Example 2B).

Fig. 3A shows corneal blink response in centrally de-epithelialized rabbit corneas treated with topical 0.01 mM (open circles), 0.1 mM (open diamonds) or 1.0 mM tetrodotoxin (open squares). Fig. 3B shows the corneal blink response for the fellow, left eyes treated with the vehicle only control.

As seen in Fig. 3A, tetrodotoxin induced local anesthesia of de-epithelialized corneas varied as a function of dose. At 2 hours after tetrodotoxin application, all of the tetrodotoxin concentrations showed some anesthetic effect. At 4 hours, rabbit eyes that were treated with 1.0 mM and 0.1 mM tetrodotoxin were significantly different, with mean anesthesia scores of 1.00 (SD=0.00, P=0.0011) and 1.67 (SD=0.52, P=0.0011), respectively. At 6 hours after tetrodotoxin administration, 5 of 6 rabbit eyes treated with 1.0 mM tetrodotoxin were anesthetic with a mean response score of 1.50 (SD=0.84, P=0.0076). By 8 hours the mean response score for 1.0 mM tetrodotoxin was 2.50 (SD=0.84), with 2 of 6 rabbits showing significantly reduced sensation.

The rabbits were observed for changes in feeding habits, movement, respiration and alertness for 24 hours, with no apparent changes observed.

The effectiveness of tetrodotoxin produced by repeated dosing was determined, as described in Example 2C. Tetrodotoxin at a concentration of 1 mM was administered every 6 hours to the centrally de-epithelialized cornea of six rabbits for 24 hours. Corneal sensation was measured every 3 hours for 30 hours.

The results, plotted in Fig. 4, show that at 3 hours after the first dose of tetrodotoxin (at t=0) all of the rabbit eyes were anesthetic with a mean response score of 1.00 (SD=0.00, P=0.0011). All six rabbit eyes remained anesthetic for the duration of the experiment, with the 40 μ l dose of 1 mM tetrodotoxin administered every six hours as indicated by the arrows in the figure. The mean response scores range from 1.00 to 1.17 (P=0.0011) over the 24 hour period of administration. At 6 hours after the final dose of tetrodotoxin (30 hours after the initial dose), 6 of 6 rabbit eyes were still anesthetic with a mean response score of 1.17 (SD=0.41, P=0.0011). At 10 hours after the final dose (34 hours after the initial dose), 5 of 6 rabbit eyes had normal sensation with a mean response score of 2.83 (SD=0.41).

The results presented in Figs. 3 and 4 show that tetrodotoxin is an effective anesthetic at a dosage provided by the 1 mM formulation in partially de-epithelialized corneas. The dose required for effective local anesthesia is significantly reduced in de-epithelialized tissues compared to intact corneas, as evidenced by comparing Fig. 1A with Fig. 3. The effective dose administered from the 1 mM formulation in rabbits is approximately 1% of the lethal human dose, as discussed in more detail below.

The studies also show that tetrodotoxin administered every 6 hours for 24 hours from formulation having a 1 mM concentration of tetrodotoxin produced local anesthesia for greater than 30 hours and that, at this dosing frequency, a reduction in corneal sensation was observed for 30 hours.

C. Re-epithelialization of Tissue

As discussed above, ocular pain after a photorefractive keratectomy procedure is severe for 24-48 hours and characterized by throbbing, watering and foreign body sensations. Conventional treatment includes topical application of non-steroidal anti-inflammatory drugs, low dose topical anesthetics, oral analgesics or bandage soft contact lens. See R. Lim-Bon-Siong, et al., Efficacy and Safety of the ProTek (Vifilcon A) Therapeutic Soft Contact Lens After Photorefractive Keratectomy, Am. J. Ophthalmology, 125:169-176 (1998). It has been shown that frequently applied topical anesthetics inhibit corneal re-epithelialization (Rosenwasser).

To determine the effect of tetrodotoxin on corneal re-epithelialization, 1 mM tetrodotoxin was administered to rabbit corneas having an epithelial defect. The epithelial defect was created in the right eye of 12 rabbits using n-heptanol soaked filter paper discs, as described in Example 3. The rabbits were randomized into two test groups for treatment with 40 µl of 1 mM tetrodotoxin every 8 hours or for no treatment. The size of the defect was measured in both test groups at regular intervals after creation of the defect. The results are shown in Fig. 5.

As seen in Fig. 5, corneal defects of corneas left untreated and of corneas treated with tetrodotoxin were essentially identical in their rates of healing. By 49 hours, 4 of 6 untreated and 3 of 6 tetrodotoxin-treated corneas were completely healed. At 56 hours, 4 of 6 untreated and 4 of 6 tetrodotoxin-treated corneas were

completely healed. By 66 hours, all but one cornea from each group had completely healed.

To determine if tetrodotoxin treatment had altered corneal thickness, pachometry was performed 90 hours after the initial wound was created. There was
5 no statistical difference in corneal thickening between untreated and tetrodotoxin-treated corneas.

This experiment shows that topical tetrodotoxin administered every 8 hours had no effect on corneal re-epithelialization compared to untreated control rabbit eyes. The rate of healing was nearly identical for the two groups, and tetrodotoxin had no
10 apparent effect on corneal thickness compared to untreated control eyes.

III. Method of Administration

As discussed above, the present invention provides a method of producing local anesthesia in a subject experiencing pain in an epithelial tissue region, typically
15 an epithelial tissue region that is damaged or is partially or completely absent, by administering a therapeutically effective dose of tetrodotoxin.

Tetrodotoxin is a nonprotein neurotoxin which is found in multiple diverse animal species, including puffer fish, porcupine fish, goby fish, newts, frogs and the blue-ringed octopus (Bower). Tetrodotoxin and its anesthetic properties have been
20 reviewed (Blakenship, Kao, Ogura).

In the experiments described above, tetrodotoxin was administered topically to the cornea of rabbit eyes and found to provide long-acting pain relief with no signs of systemic or local toxicity.

More generally, the method of the present invention is intended for use in
25 providing local anesthesia for pain associated with any epithelial tissue region in a subject, for example, pain associated with epithelial ulcers, such as a canker sore or genital lesions. Canker sores can occur alone or in groups on the inside of the cheek or lip or underneath the tongue. Severely affected people have continuously recurring ulcers which last for one to two weeks (Clayman). Long-acting, topical painkillers,
30 such as tetrodotoxin, can ease the pain without significantly retarding healing of these oral lesions.

Genital ulcers are usually caused by sexually transmitted diseases, including herpes and syphilis. The early stages of syphilis are characterized by a hard chancre,

a painful ulcer where bacteria has penetrated the skin. This may be followed by shallow, elongated ulcers once the chancre has healed. Such ulcers are painful. Genital ulceration may also be a side effect of drugs taken orally or caused by solutions applied to genital warts. Tetrodotoxin administered in accordance with the method of the invention provides relief from the pain associated with such ulcers.

Pain in epithelial tissue is also caused by burns. Burns affecting the epidermal layer are usually associated with pain, restlessness and fever. Treatment of such a burn in accordance with the method of the invention can provide relief from the attendant pain, while allowing epithelial healing.

Pain as a result of damage to or loss of epithelial tissue is also associated with other conditions and procedures, such as sore throats and plastic surgery, for example carbon dioxide laser surgery to remove for skin resurfacing and removal of wrinkles (Rosenberg), burns, genital lesions, upper or lower gastrointestinal bronchoscopy or endoscopy, intubation, dermatologic abrasions or chemical skin peels. Tetrodotoxin administered in accordance with the method of the invention is beneficial in relieving pain associated with such damaged tissues without significantly retarding healing.

A. Tetrodotoxin and Saxitoxin Formulations and Dosages

For use in the eye, tetrodotoxin and saxitoxin are typically administered in an aqueous solution, which may be an aqueous suspension, an ointment, a gel or an aqueous polymer solution. Preferably, the solution has a pH of between 4-8, more preferably between about 5-7.5. Such ophthalmic formulations suitable for topical and intraocular administration can be formulated in accordance with techniques known to those of skill in the art.

Typically, the active ingredient tetrodotoxin or saxitoxin is formulated into purified water or a physiological saline solution as a major vehicle. However, it will be appreciated that the ophthalmic formulation can contain other components, including, but not restricted to, buffering means to maintain or adjust pH, such as acetate buffers, citrate buffers, phosphate buffers and borate buffers; viscosity increasing agents such as polyvinyl alcohol, celluloses, such as hydroxypropyl methyl cellulose and carbomer; preservatives, such as benzalkonium chloride, chlorobutanol, phenylmercuric acetate and phenyl mercuric nitrate; tonicity adjustors, such as sodium chloride, mannitol and glycerine; and penetration enhancers, such as glycols.

oleic acid, alkyl amines and the like. The addition of a vasoconstrictor to the formulation is also contemplated. Combination formulations comprising said long-acting sodium channel blocking compound and an antibiotic, a steroidal or a non-steroidal anti-inflammatory drug are also contemplated.

5 The ophthalmic formulation may also be a suspension of particles, such as polymer particles or liposomes, for entrapping the active compound. For example, polymer particles prepared by suspension or emulsion polymerization to have a particle size of below about 50 μm , for entrapping the active compound or for increasing viscosity can be added to the formulation.

10 The final ophthalmic formulations in unit dosage form are preferably stored in opaque or brown containers to protect them from light exposure. Multi-dose containers can also be used, if desired, particularly if the final formulation has a low viscosity to permit constant, accurate dosages to be administered dropwise to the eye.

 The method of the invention, more generally, provides local anesthesia to
15 mucous membranes or damaged, *e.g.*, abraded, skin by topical administration of tetrodotoxin or saxitoxin in the form of solutions, creams, ointments, gels, aerosols or the like. Such formulations are prepared using ingredients and according to procedures known to those of skill in the art or as described in, for example, REMINGTON'S PHARMACEUTICAL SCIENCES.

20 Administration of tetrodotoxin has been well studied in a number of animal species (Kao), and the lethal oral dose in humans has been estimated to be about 10-18 $\mu\text{g/kg}$ (Kao). For a 70 kg person, the lethal dose would therefore be 0.7-1.26 mg.

 In the experiments performed in support of the present invention, described above, a 40 μl aliquot of 0.01 mM, 1 mM or 10 mM tetrodotoxin was administered
25 topically to rabbits' eyes. This corresponds to a dosage of between 0.127-127 μg of tetrodotoxin, well below the lethal oral human dose and giving a sufficient safety margin to allow for any differences in systemic absorption between topical and oral administration. In additional experiments, 20 μl aliquots of 0.1 mM and 0.2 mM tetrodotoxin were administered topically to partially de-epithelialized rabbit eyes,
30 resulting in 6 and 8 hours of local anesthesia respectively.

 For administration to the eye, the tetrodotoxin formulation, as a concentration of between 0.001-10 mM and in the form of aqueous solution, suspension, ointment or the like, is administered dropwise to the eye. A single drop typically administers

between 10-50 μ l. The drop volume and solution concentration, of course, determine the dosage delivered, which, for the ranges specified here, is between about 0.003-160 μ g.

5 Saxitoxin (1 mM) was administered in a 20 μ l dose to partially de-epithelialized rabbit corneas, resulting in 4 hours of local anesthesia. This dosage of saxitoxin corresponds to approximately 5.9 μ g of saxitoxin, well below the estimated human lethal dose (oral) of between 300 μ g to 1.0 mg. See Bower et al., Clin. Toxicol., 18(7):813-863 (1981).

10 For administration to the eye, the saxitoxin formulation, as a concentration of between 0.001-10 mM and in the form of aqueous solution, suspension, ointment or the like, is administered dropwise to the eye. A single drop typically administers between 10-50 μ l. The drop volume and solution concentration, of course, determine the dosage delivered, which, for the ranges specified here, is between about 0.003 to 149 μ g.

15 It will be appreciated that the dose and concentration of tetrodotoxin or saxitoxin administered is determined on an individual basis, with consideration given to such factors as age and body weight of the patient, as well as to the route of administration and the clinical anaesthetic requirements.

20 Preferably, tetrodotoxin or saxitoxin is administered topically to the painful epithelial tissue region by application of a formulation having a tetrodotoxin or saxitoxin concentration of between about 0.001-10 mM. The actual dosage of tetrodotoxin or saxitoxin administered will, of course, depend on the amount of formulation applied and the surface area over which it is applied.

25 The dosing regimen is selected to provide pain relief as needed for a particular clinical condition. Often, pain is most intense in the first 24-72 hours following damage to the tissue, by a surgical procedure or other trauma, and administration every 6 hours during this time period is effective to provide a long-acting anesthetic effect. This was demonstrated in the experiment discussed above where tetrodotoxin was administered every 6 hours for 24 hours to achieve greater than 30 hours of local
30 anesthesia in rabbits, with no signs of irritation or toxicity.

 It will be appreciated that topical administration of either tetrodotoxin or saxitoxin may be combined with conventional modes of pain relief, such as administration of oral or topical non-steroidal anti-inflammatory drugs or antibiotics

and the use of bandage soft contact lens in ocular applications. For example, methods of the invention include applying to the corneal surface of an eye of a mammal, a bandage contact lens, wherein said lens is capable of delivering an ophthalmically effective dose of said long-acting sodium channel blocking compound to said corneal surface. One of skill in the art will appreciate that there are a number of ocular drug delivery systems and methods that can be used with the present invention, some of which are described in Lee, V. and Robinson, J.R., Review: Topical Ocular Drug Delivery: Recent Developments and Future Challenges, J. Ocular Pharmacol. 2(1):67-108 (1986) (incorporated herein by reference).

Moreover, another preferred method of producing anesthesia in an eye of a mammal comprises topically administering to the corneal surface of an eye of a mammal an ophthalmically effective dose of proparacaine or other comparable local anesthetic, including tetracaine or benoxinate, in an ophthalmically acceptable vehicle before administering to said eye, an ophthalmically effective dose of a long-acting sodium channel blocking compound in an ophthalmically acceptable vehicle. Prior administration of proparacaine or other comparable local anesthetic, to the eye of a mammal effectively diminishes the production of tears by the eye, preparing the eye for topical administration of a long-acting sodium channel blocking compound, such as TTX or STX. By decreasing tearing and anesthetizing the corneal surface of an eye, the prior administration of proparacaine, tetracaine or benoxinate, decreases the amount of the long-acting sodium channel blocking compound that would wash away with tears. Thus, the method enables administration of lower effective doses of the long-acting sodium channel blocking compound. For example, dosages of proparacaine range from 0.05% to 0.5%, and preferably, from 0.05 to 0.1% formulations in any ophthalmically acceptable vehicle.

From the foregoing, it can be appreciated how various features and objects of the invention are met. The method of the invention provides an effective, long-lasting local anesthesia by topical administration of a long-acting sodium channel blocking compound to a painful epithelial tissue region. The studies reported herein illustrate, using a rabbit model, that a single dose of tetrodotoxin at a concentration of 1 mM or 10 mM achieves local anesthesia with rapid onset and for at least 8 hours. A repeated dosing regimen is effective to produce local anesthesia for greater than 30 hours. Importantly, no evidence of either ocular or systemic toxicity in the test animals was

observed at any of the dosage levels, including 10 mM tetrodotoxin. Moreover, a single dose of saxitoxin at a concentration of 1mM achieves local anesthesia upon administration to a partially abraded cornea with rapid onset and for at least 4 hours.

One of skill in the art will appreciate that any of the long-acting sodium
5 channel blocking compounds can be used according to the methods and procedures described herein to determine pharmaceutically or ophthalmically effective doses and other aspects of the invention.

VI. Examples

10 The following examples illustrate the methods and compositions of the invention, but are in no way intended to limit the invention.

Materials: Tetrodotoxin was purchased from Sigma Chemical Co. (St. Louis, MO) in vials containing 1 mg tetrodotoxin and approximately 5 mg sodium citrate buffer, pH=4.3, in lyophilized form.

15 Saxitoxin and additional tetrodotoxin was purchased from Alexis Chemicals.

EXAMPLE 1

Administration of tetrodotoxin to healthy rabbit eyes

20 A. 0.1 mM, 1 mM and 10 mM Tetrodotoxin

Tetrodotoxin formulations of 0.1 mM, 1 mM and 10 mM were prepared in a 60 mM sodium citrate carrier at pH 4.3.

Eighteen New Zealand white rabbits were divided into three groups of six rabbits. Each rabbit received a 40 μ l aliquot of one of the tetrodotoxin formulations
25 into the inferior conjunctival cul-de-sac of the right eye, with the left fellow eye receiving 40 μ l of the pH 4.3 60 mM sodium citrate carrier as a control.

Corneal sensation was tested with a 4-0 silk suture mounted upon a wooden cotton tip applicator such that the suture extended 5 mm beyond the wooden end of the applicator. The cornea was mechanically stimulated centrally three times with the
30 suture to produce grossly visible indentation of the cornea as the endpoint (similar to previous rabbit model of corneal anesthesia reported in Maurice DM, Singh T., The absence of corneal toxicity with low-level topical anesthesia. Am J Ophthalmol. 99:691-696. (1985)). Care was taken not to stimulate the eyelashes. The rabbit's

response was graded in the following fashion: no blink=1; partial blink without full eyelid closure=2; full blink=3. Thus, a score of 3 indicates full responsiveness and a score of 1 indicates full local anesthesia. The highest anesthesia score of the 3 tests was recorded for each time point.

- 5 Corneal sensation was tested prior to administration of drugs and again at 1 minute, 1 hour, 4 hours and 8 hours. The results are tabulated in Table 1 below and shown in Figs. 1A-1B. . Statistical analysis was by the non-parametric Wilcoxon test with a statistical significance of $p < 0.05$.

10

Table 1

Drug Treatment	Mean Blink Response (N = 6)				
	0 min.	1 min.	60 min.	240 min.	480 min.
0.1 mM tetrodotoxin	2.83 (0.41)	2.67 (0.52)	2.67 (0.82)	3.00 (0.00)	2.83 (0.41)
0.1 mM tetrodotoxin/control	2.83 (0.41)	3.00 (0.00)	3.00 (0.00)	3.00 (0.00)	3.00 (0.00)
1 mM tetrodotoxin	2.83 (0.41)	1.17 (0.41)	1.50 (0.84)	2.83 (0.41)	2.83 (0.41)
1 mM tetrodotoxin/control	2.83 (0.41)	2.83 (0.41)	2.83 (0.41)	3.00 (0.00)	2.67 (0.52)
10 mM tetrodotoxin	3.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.17 (0.41)	2.00 (0.89)
10 mM tetrodotoxin/control	3.00 (0.00)	3.00 (0.00)	2.83 (0.41)	3.00 (0.00)	3.00 (0.00)

*numbers in parenthesis are standard deviation of mean blink response for n=6.

B. Comparative Study: 1 mM, 10 mM tetrodotoxin and proparacaine

- 15 Eighteen New Zealand white rabbits were divided into three groups of six rabbits. Each rabbit received a 40 μ l aliquot into the inferior conjunctival cul-de-sac of the right eye of 1 mM or 10 mM tetrodotoxin or of proparacaine 0.5% (Ophthetic). The left fellow eye received 40 μ l of a placebo, control vehicle (60 mM, pH 4.3 sodium citrate).

- 20 Following the procedure described in Example 1A, the anesthetic duration was determined by measuring corneal blink response in the test rabbits at 0 min, 1 minute, 1 hour, 3 hours and 5 hours. The results are tabulated in Table 2 and shown in Figs. 2A-2B.

Table 2

Drug Treatment	Mean Blink Response (N = 6)				
	0 min.	1 min.	60 min.	180 min.	300 min.
Proparacaine	3.00 (0.00)	1.00 (0.00)	2.50 (0.84)	3.00 (0.00)	3.00 (0.00)
Proparacaine/control	3.00 (0.00)	3.00 (0.00)	2.50 (0.84)	3.00 (0.00)	3.00 (0.00)
1 mM tetrodotoxin	3.00 (0.00)	2.17 (0.75)	2.00 (0.89)	2.50 (0.55)	2.83 (0.41)
1 mM tetrodotoxin/control	3.00 (0.00)	3.00 (0.00)	2.83 (0.41)	3.00 (0.00)	3.00 (0.00)
10 mM tetrodotoxin	3.00 (0.00)	1.00 (0.00)	1.00 (0.00)	1.33 (0.82)	1.83 (0.98)
10 mM tetrodotoxin/control	3.00 (0.00)	3.00 (0.00)	3.00 (0.00)	3.00 (0.00)	3.00 (0.00)

*numbers in parenthesis are standard deviation of mean blink response for n=6.

- 5 The extent and duration of local anesthesia after topical TTX was administered to intact rabbit corneas varied as a function of dose (Figure 1A, Table 1). At 0.1 mM TTX, only partial local anesthesia was produced in 2 of 6 rabbits. At 1 mM TTX, local anesthesia initially was produced in 6 of 6 animals but the effect was generally short-lived. At one minute after administration of TTX, the mean
- 10 anesthesia score was 1.17 (SD=0.41). At 1 hour the score was 1.50 (SD=0.84) and by 3 hours the mean score had increased to 2.83 (SD=0.41). At 6 hours the TTX-vehicle had a better anesthetic score than the 1mM TTX treated eyes. However, neither of these scores were significantly different from each other or from the pretreated eyes anesthesia score of 3.0. (SD=0).
- 15 At 10mM, TTX produced a more reproducible and longer lasting local anesthesia. At one minute after administration, all rabbit corneas were anesthetic with a mean anesthesia score of 1.00 (SD=0). At 4 hours, local anesthesia was still present with a mean score of 1.17 (SD=0.41). As late as 8 hours, 4 of 6 rabbits showed some residual local anesthesia with a mean score of
- 20 2.00 (SD=0.89). This was significantly different, (P=0.0325), from the mean score of 3.00 (SD=0) obtained with vehicle alone at 8 hours (Table 1).

The anesthetic duration of 10 mM TTX was compared to that of 1 mM TTX, and proparacaine (Figure 2, Table 2). At the 10 mM dose, TTX produced significantly longer local anesthesia than proparacaine. While proparacaine produced

local anesthesia in 6 of 6 rabbits at 1 minute, by 1 hour the mean score had increased to 2.50 (SD=0.84), and at 3 hours, all eyes receiving proparacaine had normal sensation. As late as 5 hours, 4 of 6 rabbits receiving TTX showed some residual local anesthesia with a mean score of 1.83 (SD=0.98). This was significantly different, (P=0.0325), from the mean score of 3.00 (SD=0) obtained with proparacaine or vehicle alone at 5 hours (Table 2).

C. Pachometry

To evaluate whether endothelial function was significantly affected by administration of 10 mM or 1 mM TTX, pachometry readings were made (Humphrey Pachometer, Humphrey Instr., San Leandro, CA) prior to and 24 hours after TTX administration. The results are given in Table 3 as mean corneal thickness, with the standard deviation indicated in parenthesis. Pachometry readings on rabbits receiving the highest doses of TTX showed no evidence of corneal thickening during the 24 hour observation period.

Table 3

Drug Treatment	Corneal Thickness (mm)	
	0 hrs.	24 hrs.
proparacaine	0.38 (0.03)	0.38 (0.04)
proparacaine/control	0.39 (0.04)	0.38 (0.03)
1 mM tetrodotoxin	0.39 (0.04)	0.39 (0.04)
1 mM tetrodotoxin/ control	0.36 (0.01)	0.39 (0.04)
10 mM tetrodotoxin	0.38 (0.03)	0.37 (0.01)
1 mM tetrodotoxin/ control	0.36 (0.01)	0.38 (0.04)

*numbers in parenthesis are standard deviation of mean corneal thickness for n=6.

Drug treatments labelled “/control” represent fellow eyes of either proparacaine or TTX-treated eyes, wherein the fellow eyes were treated with the citrate vehicle alone.

Slit Lamp Examination and Fluorescein Staining.

Slit lamp biomicroscopy with a portable slit lamp (Kowa SL-5, Kowa Company, Japan) was performed with and without fluorescein stain from impregnated

strips moistened with balance salt solution at 12 and 24 hours after topical administration.

To assess whether TTX administration caused clinical alterations in the cornea, all animals were examined with a slit lamp after fluorescein staining. Despite the acidic vehicle, TTX administration did not cause any apparent ocular irritation after administration. There was no obvious discomfort in any of the rabbits evidenced by prolonged eye closure or repetitive blinking. No ocular injection was noted during the 24 hour observation period. At 3 hours most of the rabbits had a mild central punctate epithelial keratopathy in the area of corneal sensation testing, but there was no difference between rabbits receiving proparacaine and TTX. By 24 hours, all signs of epithelial damage had disappeared by slit lamp examination and fluorescein staining.

Toxicity

The rabbits were observed for changes in feeding habits, movement, respiration and alertness during the first 24 hours by the experimenters and for the subsequent week daily by animal care personnel.

No rabbit had any alterations of feeding, movement, respiration, or alertness during this period that suggested a toxic effect of the TTX. No rabbit died or was noted to have abnormalities in behavior by the animal care personnel for 7 days subsequent to TTX administration.

EXAMPLE 2

Topical administration of tetrodotoxin to partially de-epithelialized rabbit corneas

A. Corneal Abradement and Blink Response Test

After general anesthesia and topical application of 0.5% proparacaine to each eye, a #69 Beaver blade was used to create a central epithelial defect which measured between 3.0-3.5 mm diameter in both eyes of each test rabbit.

Corneal sensation was tested with a 4-0 silk suture mounted upon a wooden cotton tip applicator such that the suture extended 5 mm beyond the wooden end of the applicator. Because the central cornea was often rendered largely anesthetic following mechanical epithelial debridement, the cornea was stimulated in the mid-

peripheral cornea, outside of the abraded area, with the suture to produce grossly visible indentation of the cornea as the endpoint. Care was taken not to stimulate the eyelashes. The rabbit's response was graded in the following fashion; no blink=1, partial blink without full eyelid closure=2, full blink=3.

5

B. Dose Response

Tetrodotoxin in a pH 4.3 sodium citrate vehicle was formulated into concentrations of 1 mM, 0.1 mM, and 0.01 mM.

New Zealand white rabbits were divided into three experimental groups of six. Each rabbit received a 40 µl aliquot of tetrodotoxin at a concentration of 0.01 mM, 0.1 mM or 1 mM into the inferior conjunctival cul-de-sac of the right eye. A 40 µl of a pH 4.3 sodium citrate vehicle as a control into the fellow, left eye.

Corneal sensation was tested prior to administration of tetrodotoxin and after administration at 2, 4, 6 and 8 hours. The results, presented as the mean score for the 6 rabbits in each test group, are tabulated below in Table 4 and shown in Figs. 3A-3B, where Fig. 3A shows the response scores for eyes treated with tetrodotoxin and Fig. 3B shows the response scores for the fellow, control-vehicle treated eyes. Statistical analysis using the Wilcoxon test was performed by comparing the tetrodotoxin treated eyes to the pre-operative anesthesia response score.

20

Table 4

Drug Treatment	Mean Blink Response (N = 6)			
	2 hrs.	4 hrs.	6 hrs.	8 hrs
1.0 mM tetrodotoxin	1.00 (0.00)	1.00 (0.00)	1.50 (0.84)	2.50 (0.84)
1.0 mM tetrodotoxin/ control	2.83 (0.41)	3.00 (0.00)	3.00 (0.00)	3.00 (0.00)
0.1 mM tetrodotoxin	1.17 (0.41)	1.67 (0.52)	2.83 (0.41)	3.00 (0.00)
0.1 mM tetrodotoxin/ control	2.83 (0.41)	3.00 (0.00)	3.00 (0.00)	3.00 (0.00)
0.01 mM tetrodotoxin	1.50 (0.84)	2.33 (0.82)	2.83 (0.41)	2.67 (0.52)
0.01 mM tetrodotoxin/ control	2.50 (0.84)	2.67 (0.52)	3.00 (0.00)	3.00 (0.00)

*numbers in parenthesis are standard deviation of mean blink response for n=6.

TTX-induced local anesthesia of de-epithelialized corneas varied as a function of dose. At 2 h after TTX application, all of the TTX concentrations showed some anesthetic effect. TTX at 1.0 mM, 0.1 mM, and 0.01 mM had mean anesthesia scores of 1.00 (SD=0.00, P=0.0011), 1.17 (SD=0.41, P=0.0076), and 1.50 (SD=0.84, P=0.0076) respectively. At 4 h, rabbit eyes that were treated with 1.0 mM and 0.1 mM TTX were still significantly anesthetic with mean anesthesia scores of 1.00 (SD=0.00, P=0.0011) and 1.67 (SD=0.52, P=0.0011), respectively. In contrast, the mean anesthesia score of rabbits treated with 0.01 mM TTX was returning to normal at 2.33 (SD=0.82) by 4 h. By 6 h, rabbit eyes treated with either 0.1 mM or 0.01 mM had mean scores of 2.83 (SD=0.41). At 6 h after TTX administration, five of six rabbit eyes treated with 1.0 mM TTX were still partially anesthetic with a mean anesthesia score of 1.50 (SD=0.84, P=0.0076). By 8 h the mean anesthesia score for 1.0 mM TTX was approaching normal at 2.50 (SD=0.84), with only two of six rabbits showing any anesthetic effect.

15

C. Dosing Frequency

Experiments were conducted to test whether TTX could produce prolonged effectiveness with repeated dosing. A 40 μ l aliquot of 1 mM tetrodotoxin was administered every 6 hours for 24 hours to the centrally de-epithelialized cornea of 6 rabbits. Corneal sensation was monitored, by scoring the eye for blink response as described above, every 3 hours for 24 hours and at 17 hours, 30 hours and 34 hours after the initial dose. Response scores after administration of tetrodotoxin were compared to pre-operative response scores for statistical analysis by the Wilcoxon test. The results are tabulated in Table 5 and shown in Fig. 4, where the arrows in the figure indicate the dosage times.

25

Table 5

Time (hours)	Blink Response
0	3.00 (0.00)
3	1.00 (0.00)
6	1.17 (0.41)
9	1.00 (0.00)
12	1.00 (0.00)
15	1.00 (0.00)
18	1.00 (0.00)
21	1.17 (0.41)
24	1.00 (0.00)
17	1.00 (0.00)
30	1.17 (0.41)
34	2.83 (0.41)

*numbers in parenthesis are standard deviation of mean blink response for n=6.

- 5 At 3 hours after the first TTX administration, all of the rabbit eyes were anesthetic with a mean anesthesia score of 1.00 (SD=0.00, P=0.0011). All six rabbit eyes remained anesthetic for the duration of the experiment, with mean anesthesia scores ranging from 1.00 to 1.17 (P=0.0011 throughout). At 6 h after the final TTX administration and 30 h after the initial TTX administration, six of six rabbit eyes
- 10 were still anesthetic with a mean anesthesia score of 1.17 (SD=0.41, P=0.0011). At 10 h after the final TTX administration and 34 h after the initial TTX administration, five of six rabbit eyes had normal sensation with a mean anesthesia score of 2.83 (SD=0.41).

EXAMPLE 3
Administration of tetrodotoxin to
Partially de-epithelialized rabbit corneas

A. Corneal Abradement

5 To determine whether TTX inhibits corneal re-epithelialization, twelve New Zealand White rabbits were anesthetized with a mixture of ketamine and xylazine. A corneal trephine was used to make Whatman #2 filter paper discs measuring 7.5 mm in diameter. The discs were soaked in n-heptanol solution and blotted to remove excess liquid. One disc was placed on the central cornea of the right eye of each
10 rabbit for 30 seconds. After removal, the cornea was washed with balanced salt solution to remove the loosened epithelial cells. Topical fluorescein was applied and the diameter of the epithelial defect was measured in 2 meridians (12:00-6:00 and 3:00-9:00) using calipers. The radius of the defect was determined, and the defect area was calculated as previously described using an equation that corrects for the
15 curvature of the rabbit cornea. Crosson CE, Klyce SD, Beuerman RW (1986) Epithelial wound closure in the rabbit cornea. Invest Ophthalmol Vis Sci 27:464-473.

B. Effect of Multiple Doses of TTX on Re-epithelialization of Abraded Rabbit Corneas

20 The rabbits were randomized into two test groups for treatment with 40 µl of 1 mM tetrodotoxin every 8 hours or for no treatment. The rabbits were re-anesthetized with ketamine and xylazine and epithelial defect size was re-measured with calipers at 17, 32, 42, 49, 56, and 66 h after the creation of epithelial defects. Twenty-four hours after the corneal epithelial defects were closed (completely healed), pachometry
25 (Humphrey Ultrasonic Pachometer) was performed in both the experimental and fellow eyes of each rabbit.

The results are shown in Fig. 5 and Table 6.

Table 6. Corneal Defect Size

TIME (h)	AREA OF DEFECT (mm ²)	
	CONTROL	TTX
0	53.0+/-2.0	49.0+/-5.8
17	35.6+/-8.9	37.9+/-7.1
32	12.9+/-6.4	15.7+/-7.1
42	5.7+/-9.5	5.6+/-6.3
49	2.8+/-4.4	3.0+/-5.7
56	2.7+/-5.1	2.5+/-5.9
66	0.7+/-1.6	0.3+/-0.7

5

Corneal defects of untreated and 1.0 mM TTX-treated corneas were essentially identical in their healing rates. By 48 h, four of six untreated corneas and three of six TTX-treated corneas were completely healed. At 56 h, four of six untreated and four of six TTX--treated corneas were completely healed. By 66 h, all but one cornea from each group had completely healed. In these remaining eyes, only a small defect, 0.67 +/- 1.63 mm² for the TTX-treated rabbit and 0.29 +/- 0.72 mm² for the untreated control rabbit, remained

10

C. Effect of Multiple Doses of TTX on Corneal Thickness After Re-epithelialization

15

In order to determine whether TTX had an effect on corneal thickness after re-epithelialization compared to untreated control eyes, corneal thickness was measured 92 hours after creation of a corneal abrasion (OD) in rabbit eyes that had been treated with multiple doses of 1.0 mM TTX. Corneal thickness of healed corneal abrasions (OD) and uninjured fellow eyes (OS) were measured by pachometry. The results are given as the mean defect area and the standard deviation ($\bar{n}=6$)

20

Table 7

DRUG TREATMENT	CORNEAL THICKNESS	
	OD	OS
NONE	0.39+/-0.04	0.43+/-0.04
1.0 mM TTX	0.40+/-0.04	0.41+/-0.05

There was no statistically significant difference in corneal thickness between untreated and TTX-treated corneas (Table 7).

5

EXAMPLE 4

**Topical administration of Tetrodotoxin to rabbit eyes
after excimer laser keratectomy**

10 TTX in a pH 4.3 sodium citrate vehicle was used in the following experiments (Sigma Chemical Co., St. Louis, MO). New Zealand white rabbits were divided into two experimental groups, each consisting of six rabbits. After general anesthesia by intramuscular injection of a mixture of xylazine and ketamine, followed by topical application of 0.5% proparacaine (Ophthalmic, Allergan, Irvine, CA) to each right eye,
15 the rabbits underwent excimer laser keratectomy on their right eyes.

Excimer laser keratectomy was performed using the Star Excimer Laser System (VISX, Inc.) in phototherapeutic keratectomy (PTK) mode. Excimer laser keratectomy was performed to create a 5 mm diameter wound, 75 μ m in depth. The repetition rate of the laser was set at 6 Hz with a pulse energy density of 160 mJ/cm².

20 One group of six rabbits then received a 40 μ l aliquot of 1 mM TTX into the inferior conjunctival cul-de-sac of their right eyes and the other group of six rabbits received 40 μ l of the pH 4.3 sodium citrate vehicle into the inferior conjunctival cul-de-sac of their right eyes as a control. The rabbits were treated with 1 mM TTX or vehicle again at 6, 12, 18, and 24 hours.

25 Corneal sensation was tested as previously described. Briefly, sensation was tested with a 5 mm silk suture mounted on a wooden cotton tip applicator. The cornea was stimulated with the suture in the mid-peripheral cornea, outside of the excimer laser keratectomy treated area. Care was taken not to stimulate the eyelashes. The rabbit's response was graded in the following fashion: no blink=1, partial blink
30 without full eyelid closure=2, full blink=3. Corneal sensation was tested at 3, 6, 9, 12,

15, 18, 21, 24, 30, 32, and 40 hours. At 6, 12, 18 and 24 hours the corneal sensation was tested prior to re-administration of TTX or vehicle. The rabbits were observed for changes in feeding habits, movement, respiration and alertness. The data are presented in Figure 6 as the mean score of 6 rabbits/treatment group. TTX treated eyes' anesthesia scores were compared to vehicle treated eyes' anesthesia scores for statistical significance by the Wilcoxon test.

Administration of 40 μ l of 1 mM TTX every 6 hours for 24 hours after excimer laser keratectomy produced nearly complete local anesthesia for at least 30 hours with a mean anesthesia score of 1.17 (SD=0.41) ($p=0.011$, Wilcoxon test) (Fig. 6). Three hours following each application of TTX, the mean anesthesia score for TTX treated eyes was 1.0 (SD=0). Six hours following each application of TTX the mean anesthesia scores were between 1.0 (SD=0) and 1.5 (SD=0.84). At least 4/6 of the rabbits were completely anesthetic at six hours following each application of TTX. At 32 hours, 8 hours following the final application of TTX, there was still significant local anesthesia of the TTX treated corneas ($p=0.0325$, Wilcoxon test). The mean anesthesia score was 2.0 (SD=0.89). At 40 hours, 18 hours following the final application of TTX, all of the rabbits' corneas had returned to normal sensation. In contrast, after 9 hours, vehicle treated eyes all had normal sensation for the duration of the experiment (Fig. 6). There was a very slight local anesthesia of the vehicle treated eyes at 6 and 9 hours following excimer laser keratectomy, with the mean anesthesia score being 2.83 (SD=0.41). However, this was due in each case to 1/6 rabbits scoring 2, 5/6 rabbits scoring 3, and was not significant. At 3 hours following excimer laser keratectomy the vehicle treated eye's mean anesthesia score was 2.16 (SD=0.75). This was probably due to lingering effects of the general and topical anesthetics that were administered prior to the excimer laser keratectomy procedure.

EXAMPLE 5

Effect of topical administration of TTX following PRK on corneal re-epithelialization

To determine whether TTX inhibited corneal re-epithelialization following excimer laser keratectomy, topical fluorescein was applied after rabbits were given general anesthesia by intramuscular injection of a mixture of xylazine and ketamine.

The diameter of the circular epithelial defect was measured using calipers in 2 meridians (12:00-6:00 and 3:00-9:00), and the radius of the defect was calculated. The radius was used to calculate the area of the defect using the method of Crosson et al. that corrects for the curvature of the rabbit cornea (Crosson CE, et al., Invest Ophthalmol Vis Sci., 27:464-473 (1986)). General anesthesia was administered and the size of the epithelial defect was measured at 24, 40, 49, 63, 68, and 72 hours following excimer laser keratectomy. Observers were masked as to the contents of eye drops given to the rabbits.

To assess whether or not repeated administration of TTX had any effect on the rate of epithelial healing, epithelial defect area was measured over 72 hours following excimer laser keratectomy. As shown in Figure 7, there was little difference in healing rate of vehicle and TTX treated eyes. At 24 hours there was no significant difference in healing between TTX treated and vehicle treated eyes ($p > 0.05$, t-test). At 40 hours the TTX treated eyes had larger defects than vehicle treated eyes 7.85 mm² vs. 4.54 mm² ($p < 0.025$, t-test). However, at 49 hours, and thereafter, both groups were equally healed ($p > 0.05$, t-test).

Toxicity.

The rabbits involved in the corneal anesthesia and corneal wound healing experiments following excimer laser keratectomy described above were observed carefully for any signs of systemic toxicity during the course of the experiment. No rabbit had any alterations of feeding, movement, respiration, or alertness during this period that suggested a toxic effect of the TTX. No rabbit died or was noted to have abnormalities in behavior subsequent to TTX administration. Additionally, no signs of local toxicity such as ocular injection or corneal haze were apparent in any rabbit.

EXAMPLE 6**Systemic Absorption of TTX Topically Applied to Abraded Rabbit Eyes**

5 TTX (Alexis Corporation) was formulated into 20 μ l doses containing 100 μ g of TTX in a pH 4.3 citrate buffer (60 mM). Four Dutch banded rabbits were weighed. After general anesthesia by intramuscular injection of a mixture of xylazine and ketamine, followed by topical application of 0.5% proparacaine to each eye, a #69 Beaver blade was used to create a central epithelial defect which measured between 4
10 mm diameter in one eye of each rabbit. Each rabbit then received a 20 μ l aliquot of TTX into the inferior conjunctival cul-de-sac of the abraded eye. At 10 min, 20 min and 40 min following administration of TTX, 4 ml of blood was taken from each rabbit. The blood was clotted and the serum collected.

TTX was purified from serum samples using the purification scheme of
15 Yasumoto and Michishita. Fluorometric determination of tetrodotoxin by high performance liquid chromatography. Agric Biol Chem 49: 3077-3080 (1985). Briefly, 300 μ l of each serum sample was extracted by boiling for 10 min in 0.02N acetic acid, and TTX was purified by chromatography over Amberlite CG-50. TTX was eluted from the Amberlite with 0.5 N acetic acid, samples were dried in a vacuum
20 centrifuge and TTX was resuspended in 50 μ l of phosphate buffered saline.

Assays to quantify the amount of TTX purified from serum samples were performed using a tissue culture bioassay. Hamasaki, K, Kogure, K and Ohwada, K. A biological method for the quantitative measurement of tetrodotoxin (TTX): tissue culture bioassay in combination with a water-soluble tetrazolium salt. Toxicon 34:
25 490-495 (1996). Briefly, 2 to 3x10⁵ mouse Neuro 2A cells per well were plated on 96 well microtiter plates in 200 μ l of RPMI-1640 medium containing 10 fetal bovine serum and incubated overnight in a 37°C tissue culture incubator. After 24 hours the cells were given 10 μ l of the TTX samples, 20 μ l of 10 mM ouabain, and 20 μ l of 0.5 mM veratridine. After an additional 24 hours at 37°C, the cells were given 20 μ l of
30 WST-1 cell counting reagent (Dojindo Corp.) and allowed to incubate an additional 3 hours at 37°C. The yellow color produced by the WST-1 reagent was quantified by reading the absorbance at 430 nm with a reference wave length of 600 nm. All assays were performed in duplicate. TTX concentrations were derived by comparison to a standard curve generated using normal rabbit serum that had been spiked with known

amounts of pure TTX and subjected to the same purification scheme as the rabbit serum samples. The results are given in Table 8 as the average amount of TTX in μg per ml of serum at each time point in the table \pm standard deviation ($N=4$) (SE = Standard Error). The average amount of TTX absorbed into the serum is derived by assuming a serum volume equal to 3% of the mass of each rabbit. Kaplan, HM and Timmons, EH. The Rabbit: a Model of the Principals of Mammalian Physiology and Surgery. Academic Press, NY (1979).

Table 8

Time (mins)	TTX ($\mu\text{g}/\text{ml}$)	Std. Error	TTX in Serum (Total μg absorbed)	Std. Error
0	0		0	
10	0.16 \pm 0.09	(SE = 0.04)	6.39 \pm 4.61	(SE = 2.30)
20	0.33 \pm 0.16	(SE = 0.08)	14.44 \pm 6.09	(SE = 3.04)
40	0.31 \pm 0.11	(SE = 0.06)	13.92 \pm 4.06	(SE = 2.03)

Thus, after 20 minutes about 14% of the applied dose of TTX was absorbed into the serum. This persisted for at least 40 minutes.

EXAMPLE 7**Topical Application of Single Dose (20 μl) of 0.1 mM TTX to Abraded Rabbit Cornea**

TTX (Alexis Corporation) was obtained and formulated into a 0.1 mM concentration in a pH 4.3 sodium citrate vehicle (60 mM). Dutch banded rabbits were divided into two experimental groups of five and six. After general anesthesia by intramuscular injection of a mixture of xylazine and ketamine, followed by topical application of 0.5% proparacaine to each eye, a #69 Beaver blade was used to create a central epithelial defect which measured between 4 mm diameter in both eyes of each rabbit. Five rabbits received a 20 μl aliquot of 0.1 mM TTX into one eye and six rabbits received 20 μl of the pH 4.3 sodium citrate vehicle as a control into one eye. Experimenters were masked to the contents of each aliquot.

Corneal sensation was tested with a 4-0 silk suture mounted upon a wooden cotton tip applicator such that the suture extended 5 mm beyond the wooden end of the applicator. Because the central cornea was often rendered anesthetic following mechanical epithelial debridement, the cornea was stimulated in the mid-peripheral

cornea, outside of the abraded area, with the suture to produce grossly visible indentation of the cornea as the endpoint. Care was taken not to stimulate the eyelashes. The rabbit's response was graded in the following fashion; no blink=1, partial blink without full eyelid closure=2, full blink=3. A score of 3 indicates full responsiveness and a score of 1 indicates full local anesthesia. Corneal sensation was tested prior to administration of drugs and again at 4 and 6 hours. The rabbits were observed for changes in feeding habits, movement, respiration and alertness for 24 hours. The data are presented as the mean score of 6 rabbits/treatment group. TTX treated eyes' anesthesia scores were compared to their vehicle treated fellow eyes' anesthesia scores for statistical analysis by the Wilcoxon test. The results are shown in Figure 8.

At 4 hours the vehicle treated eyes had an average anesthesia score of 2.67 (SD=0.52). In contrast the TTX treated eyes were significantly anesthetic with a mean score of 1.20 (SD=0.45) ($p=0.0411$). At 6 hours the vehicle treated eyes had a mean anesthesia score of 3.00 (SD=0.00). In contrast at 6 hours the TTX treated eyes were still significantly anesthetic with a mean anesthesia score of 2.00 (SD=1.00) ($p=0.0411$). Thus, one 20 μ l dose of 0.1 mM TTX provided at least 6 hours of local anesthesia when applied to an abraded rabbit cornea.

20

EXAMPLE 8

Topical Application of Single Dose (20 μ l) of 0.2 mM TTX to Abraded Rabbit Cornea

TTX was obtained and formulated into a 0.2 mM concentration in a pH 4.3 sodium citrate vehicle (60 mM). Dutch banded rabbits were divided into two experimental groups of five and six. After general anesthesia by intramuscular injection of a mixture of xylazine and ketamine, followed by topical application of 0.5% proparacaine to each eye, a #69 Beaver blade was used to create a central epithelial defect which measured between 4 mm diameter in both eyes of each rabbit. Six rabbits received a 20 μ l aliquot of 0.2 mM TTX into one eye and five rabbits received 20 μ l of the pH 4.3 sodium citrate vehicle as a control into one eye. Experimenters were masked to the contents of each aliquot.

Corneal sensation was tested with a 4-0 silk suture mounted upon a wooden cotton tip applicator such that the suture extended 5 mm beyond the wooden end of

the applicator. Because the central cornea was often rendered anesthetic following mechanical epithelial debridement, the cornea was stimulated in the mid-peripheral cornea, outside of the abraded area, with the suture to produce grossly visible indentation of the cornea as the endpoint. Care was taken not to stimulate the eyelashes. The rabbit's response was graded in the following fashion; no blink=1, partial blink without full eyelid closure=2, full blink=3. A score of 3 indicates full responsiveness and a score of 1 indicates full anesthesia. Corneal sensation was tested prior to administration of drugs and again at 4, 6, and 8 hours. The rabbits were observed for changes in feeding habits, movement, respiration and alertness for 24 hours. The data are presented as the mean score of 6 rabbits/treatment group. TTX treated eyes' anesthesia scores were compared to their vehicle treated fellow eyes' anesthesia scores for statistical analysis by the Wilcoxon test. The results are shown in Figure 9

All of the vehicle treated eyes were normal throughout the experimental period with an average anesthesia score of 3.00 (SD=0.00). In contrast the TTX treated eyes showed significant local anesthesia. At 4 hours the TTX treated eyes were significantly anesthetic with a mean score of 1.17 (SD=0.41) ($p=0.0022$). At 6 hours the TTX treated eyes were still significantly anesthetic with a mean anesthesia score of 1.33 (SD=0.82) ($p<0.0152$). At 8 hours the TTX treated eyes were still significantly anesthetic with a mean anesthesia score of 1.50 (SD=0.84) ($p<0.0152$). Thus, one 20 μ l dose of 0.2 mM TTX provided at least 8 hours of local anesthesia when applied to an abraded rabbit cornea.

EXAMPLE 9

Calculation of Dosages

TTX has been determined to be an effective and non-toxic anesthetic for topical administration to partially de-epithelialized rabbit corneas for at least 6 h at a dose of 1.0 mM, or approximately 1.0-1.8% of the estimated lethal human dose. Even lower effective dosages for topical administration to partially de-epithelialized rabbit corneas were established, including 20 μ l aliquots of either 0.1 mM TTX or 0.2 mM TTX, corresponding to 0.6 μ g and 1.27 μ g, respectively.

While the toxic dose of TTX has been well studied in a number of animal species (Kao CY. Tetrodotoxin, saxitoxin and their significance in the study of

excitation phenomena. Pharm Rev 18:997-1049 (1966)), it has been only imprecisely estimated for humans. Cornish reported a case of TTX intoxication and estimated the lethal oral dose to be about 10-18 $\mu\text{g/kg}$ based on the amount of fish tissue consumed (Cornish JN, Susceptibility of man to pufferfish toxin. Med J Aust 2:48 (1973)). For
5 a 70-kg person, the lethal dose would therefore be 0.7-1.26 mg. The dose lethality curve for TTX is very steep. In mice, the minimum intraperitoneal lethal dose is 8 $\mu\text{g/kg}$ and the LD100 is 12 $\mu\text{g/kg}$ (Kao CY, Fuhrman FA, Pharmacological studies on tarichatoxin, a potent neurotoxin. J Pharmacol 140:31-40 (1963)). The lethal dose for oral administration in mice is much higher, 332 $\mu\text{g/kg}$ (Kao CY, Pharm Rev 18:997-
10 1049 (1966)). Because of the steep dose-lethality curve, reduction of the effective topical dose of TTX to 1% or less of the minimum lethal dose should result in a therapeutically useful dose

More sensitive testing systems using an esthesiometer, such as the Boberg-Ans or the Cochet and Bonnet device are well-known in the field and can be used to
15 determine safe human dosages. Such testing can help determine whether TTX can produce partial local anesthesia or analgesia at doses even lower than 1.0-1.8% of the estimated lethal human dose used in this study.

EXAMPLE 10

20 **Topical Administration of Saxitoxin (STX) to Partially De-epithelialized Rabbit Corneas**

Saxitoxin (Alexis Corporation) was formulated into 1 mM concentration in balanced saline solution (BSS). Ten Dutch banded rabbits were divided into two experimental groups of five. After general anesthesia by intramuscular injection of a
25 mixture of xylazine and ketamine, followed by topical application of 0.5% proparacaine to each eye, a #69 Beaver blade was used to create a central epithelial defect which measured between 4 mm diameter in one eye of each rabbit. Five rabbits then received a 20 μl aliquot of Saxitoxin into the inferior conjunctival cul-de-sac of one eye and five rabbits received a 20 μl aliquot of the BSS vehicle as a control
30 into the fellow eye. Experimenters were masked to the contents of each aliquot.

Corneal sensation was tested with a 4-0 silk suture mounted upon a wooden cotton tip applicator such that the suture extended 5 mm beyond the wooden end of the applicator. Because the central cornea was often rendered anesthetic following

mechanical epithelial debridement, the cornea was stimulated in the mid-peripheral cornea, outside of the abraded area, with the suture to produce grossly visible indentation of the cornea as the endpoint. Care was taken not to stimulate the eyelashes. The rabbit's response was graded in the following fashion; no blink=1, partial blink without full eyelid closure=2, full blink=3. A score of 3 indicates full responsiveness and a score of 1 indicates full local anesthesia. Corneal sensation was tested prior to administration of drugs and again at, 2, 4, and 6 hours. The rabbits were observed for changes in feeding habits, movement, respiration and alertness for 24 hours. The data are presented as the mean score of 5 rabbits/treatment group. The results are shown in Figure 10 (As the mean local anesthesia score +/- standard error) and again in Table 9 (as the mean anesthesia score +/- the standard deviation). Statistical analysis was by the Wilcoxon test.

Table 9

Time (min)	1 mM Saxitoxin	Vehicle
120	1.0+/-0.00	2.8+/-0.45
240	1.80+/-1.09	2.8+/-0.45
360	3.0+/-0.00	2.8+/-0.45

After 2 hours all of the Saxitoxin treated rabbit eyes were significantly anesthetic compared to vehicle treated eyes with a mean anesthesia score of 1.0 (SD=0)(p=0.004). In contrast the vehicle treated eyes had an mean anesthesia score of 2.8 (SD=0.45). After 4 hours the Saxitoxin treated eyes were still significantly anesthetic with a mean anesthesia score of 1.8 (SD=1.09)(p=0.0159), while the vehicle treated eyes had a mean anesthesia score of 2.8 (SD=0.45). By 6 hours the Saxitoxin treated eyes had returned to normal with a mean anesthesia score of 3.0 (SD=0), while the vehicle treated eyes had a mean anesthesia score of 2.8 (SD=0.45). None of the rabbits showed any signs of systemic toxicity. Thus, treatment of corneal abraded rabbit corneas with 20 μ l of 1 mM Saxitoxin provided at least 4 hours of corneal anesthesia.

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- 25 All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.
- 30 Although the invention has been described with respect to particular embodiments, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention.

WE CLAIM:

1. A method of producing local anesthesia in an epithelial tissue region of a mammal, comprising
5 topically administering an effective dose of a long-acting sodium channel blocking compound, in a pharmaceutically suitable vehicle, to said epithelial tissue region of said mammal
2. The method of claim 1, wherein said long-acting sodium channel
10 blocking compound does not inhibit re-epithelialization of said epithelial tissue region.
3. The method of claim 2, wherein said long-acting sodium channel blocking compound is a compound capable of specifically binding to a site on an
15 extracellular region of a sodium channel alpha subunit.
4. The method of claim 3, wherein said site is on an SS2 extracellular region of a sodium channel alpha subunit.
- 20 5. The method of claim 4, wherein said long-acting sodium channel blocking compound is tetrodotoxin.
6. The method of claim 4, wherein said long-acting sodium channel blocking compound is saxitoxin.
25
7. The method of claim 5, wherein said effective dose of tetrodotoxin is administered from a formulation containing tetrodotoxin at a concentration of between 0.001-10 mM.
- 30 8. The method of claim 1, wherein said administering is to a partially or completely de-epithelialized corneal tissue region.

9. The method of claim 1, wherein said administering includes instillation of drops of said sodium channel blocking compound to the eye following corneal surgery.

5 10. The method of claim 5, wherein said tetrodotoxin is administered in a vehicle having a pH of between 4-8.

11. The method of claim 10, wherein said vehicle has a pH of between 5-7.5.
10

12. The method of claim 2, wherein said sodium channel blocking compound is administered every 6-8 hours for between about 24-72 hours.

13. The method of claim 1, wherein said epithelial tissue region is a region in the upper or lower gastrointestinal tract.
15

14. The method of claim 1, wherein said epithelial tissue region is associated with genital lesions in the genital area.

20 15. The method of claim 1, where said epithelial tissue region is a facial epithelial tissue region.

16. A method of producing local anesthesia in an eye of a mammal, comprising
25 topically administering to the corneal surface of the eye of said mammal, in an ophthalmically suitable vehicle, an ophthalmically effective dose of a long-acting sodium channel blocking compound.

17. The method of claim 16, wherein said corneal surface has an epithelial layer that is partially or completely de-epithelialized.
30

18. The method of claim 16, wherein said long-acting sodium channel blocking compound is a compound capable of specifically binding to a site on an

extracellular region of a sodium channel alpha subunit, wherein said site is on either an SS1 region or an SS2 region.

19. The method of claim 18, wherein said long-acting sodium channel
5 blocking compound is tetrodotoxin and said effective dose is administered from a formulation containing tetrodotoxin at a concentration between about 0.001-10 mM.

20. The method of claim 19, wherein said long-acting sodium channel
blocking compound is tetrodotoxin and said effective dose is administered from a
10 formulation containing tetrodotoxin at a concentration between about 0.01 mM to 0.2 mM.

21. The method of claim 20, wherein said tetrodotoxin is administered in a vehicle having a pH of between about 4-8.

15 22. The method of claim 16, further comprising topically administering an ophthalmically effective dose of proparacaine to the corneal surface of the eye of said mammal, in an ophthalmically suitable vehicle, before topically administering said long-acting sodium channel blocking compound.

20 23. A method of reducing pain in a mammal following corneal refractive surgery, comprising,
topically administering to a corneal surface of an eye of said mammal, in an ophthalmically suitable vehicle, an ophthalmically effective dose of a long-acting sodium channel blocking compound.

25 24. The method of claim 23, further comprising the step of instilling drops of a non-steroidal anti-inflammatory compound in the eye of said mammal.

25 25. The method of claim 23, further comprising applying to the eye of said mammal a bandage contact lens, wherein said lens is capable of delivering said long-acting sodium channel blocking compound to said corneal surface.

26. The method of claim 23, further comprising administering to said mammal an antibiotic or a non-steroidal anti-inflammatory drug.

27. As an article of manufacture, an ophthalmically acceptable dosage
5 container comprising:

(a) from about 0.003 μg to 160. μg of tetrodotoxin in an ophthalmically acceptable vehicle at pH of between about 4-8.

28. The article of claim 27, wherein said container comprises: from about
10 0.127 μg to 2.54 μg of tetrodotoxin.

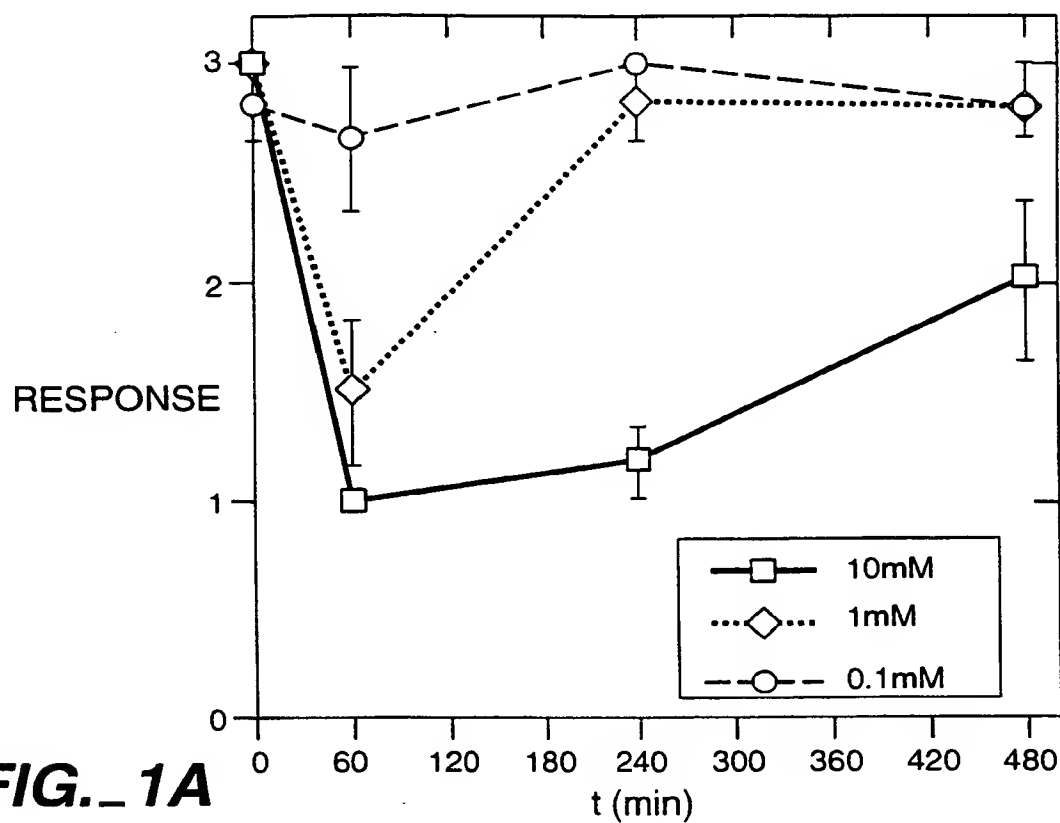
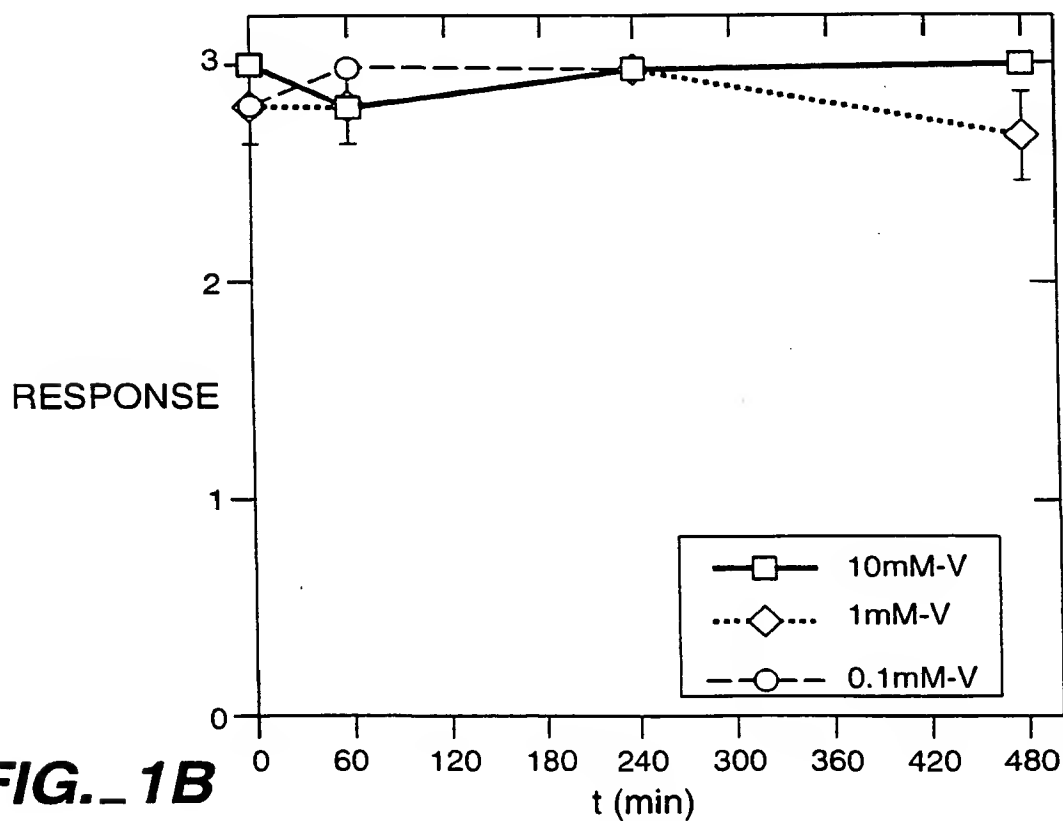
29. A local anesthetic composition which comprises an ophthalmically effective amount of tetrodotoxin, in a concentration of 0.01 mM to 1 mM, in an ophthalmically acceptable vehicle at pH 4-8.
15

30. The composition of claim 29, wherein said concentration is between 0.01 mM and 0.2 mM tetrodotoxin.

31. The composition of claim 30, wherein said concentration is between
20 0.01 mM and 0.1 mM tetrodotoxin.

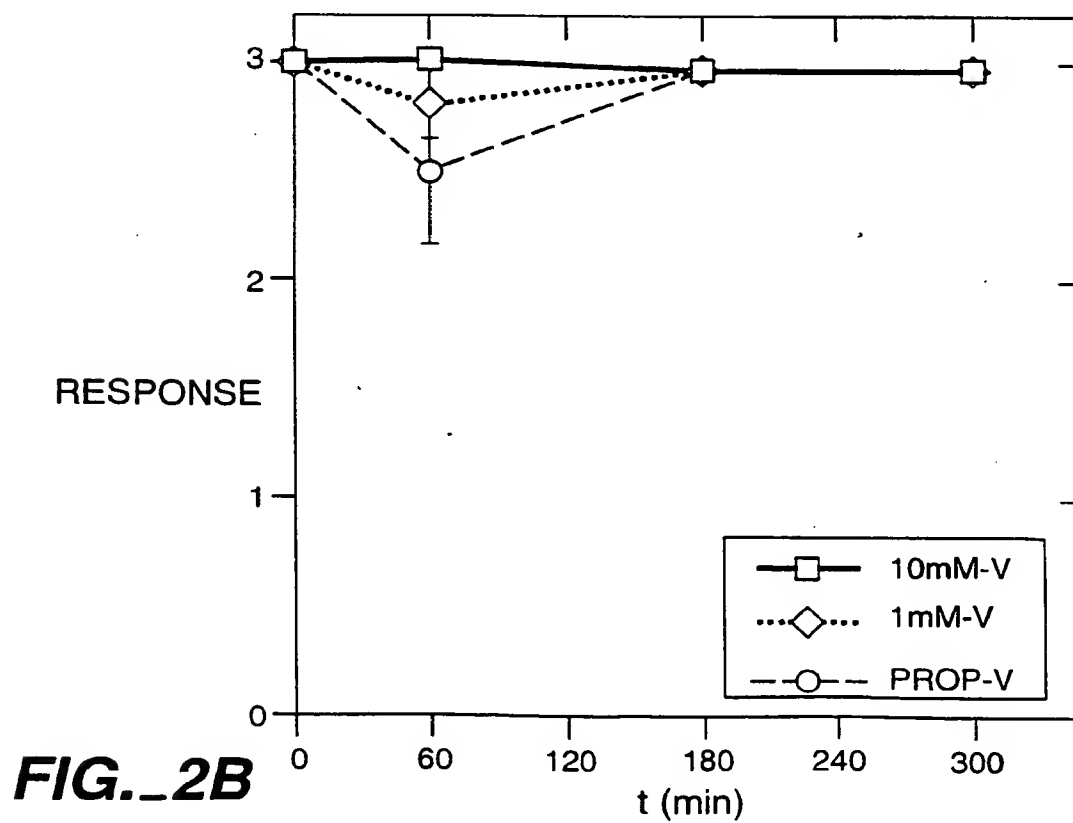
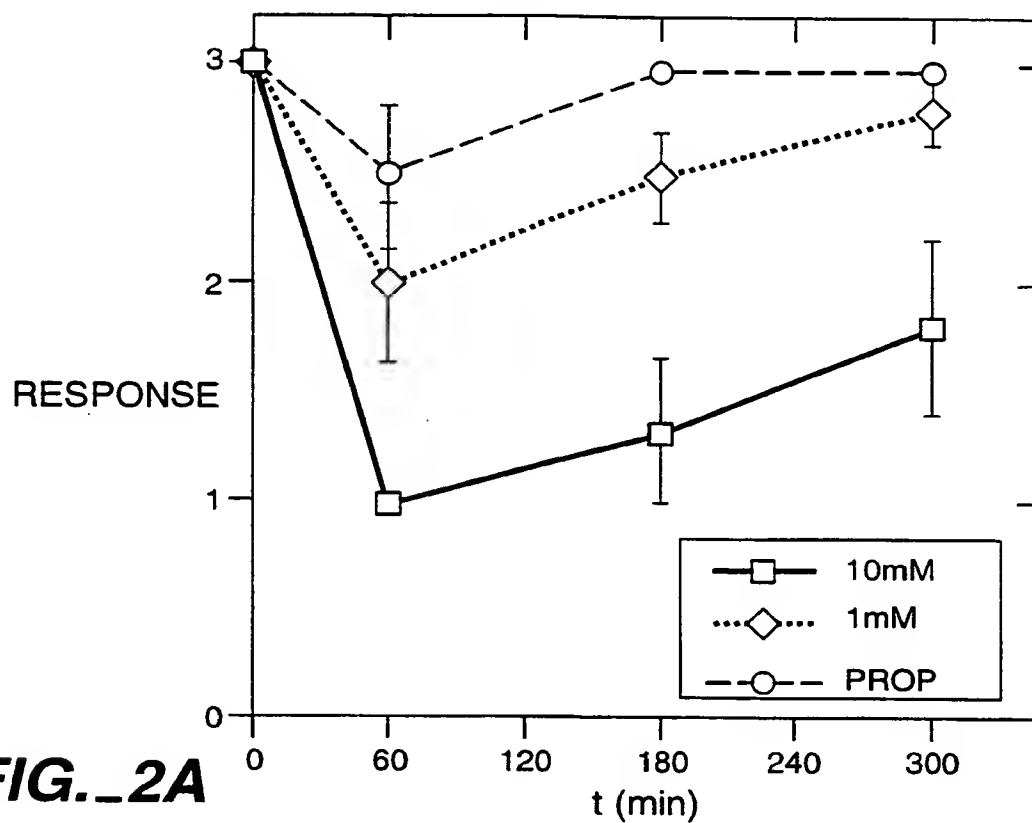
32. A local anesthetic composition which comprises an ophthalmically effective amount of saxitoxin, in a concentration of 0.01 mM to 1 mM, in an ophthalmically acceptable vehicle at pH 4-8.

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**FIG. 1A****FIG. 1B**

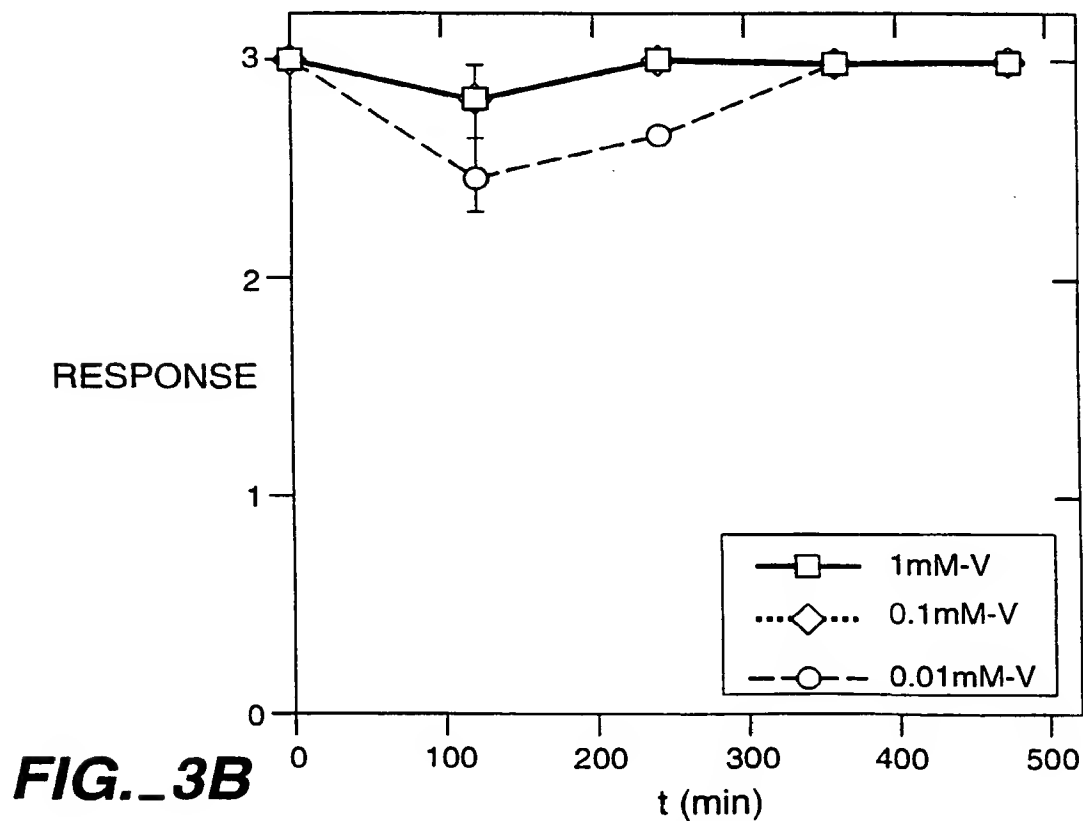
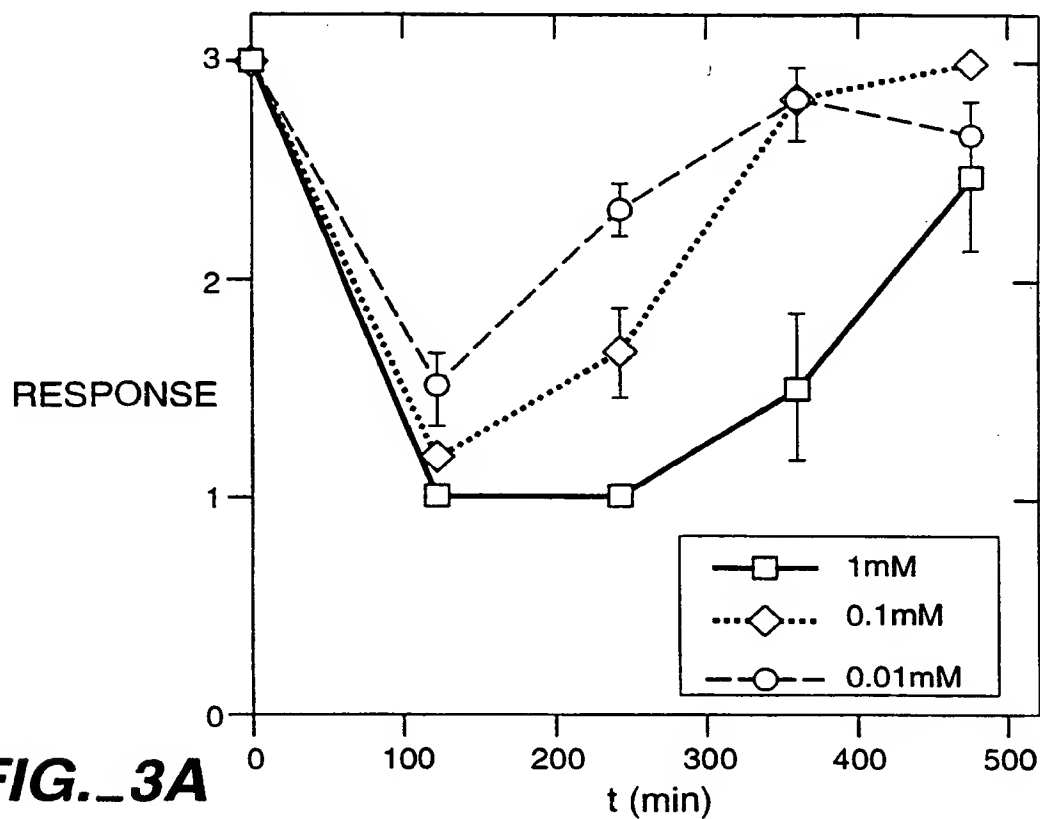
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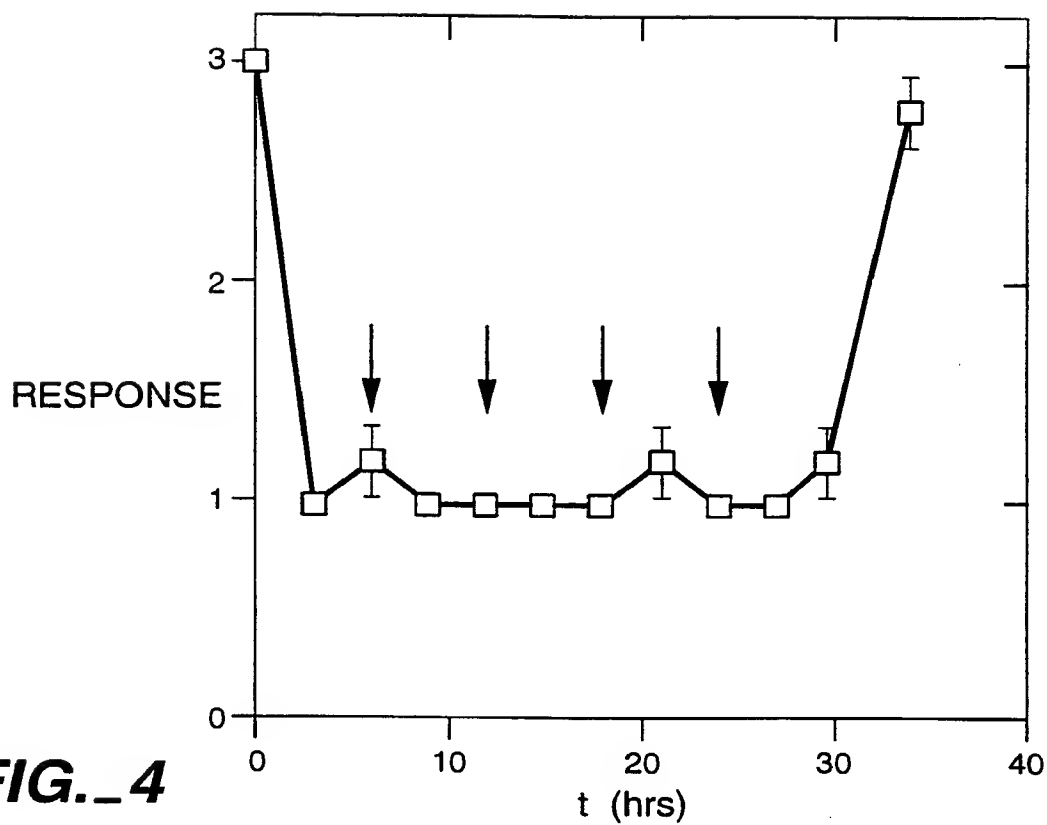
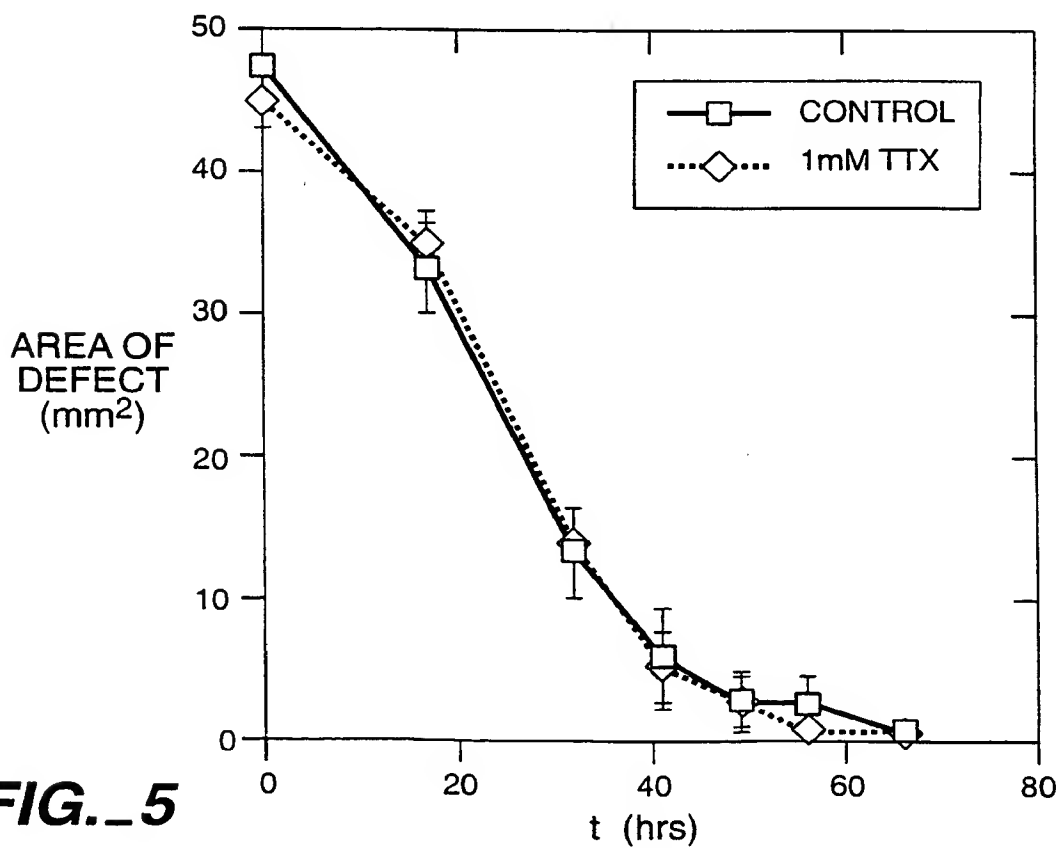


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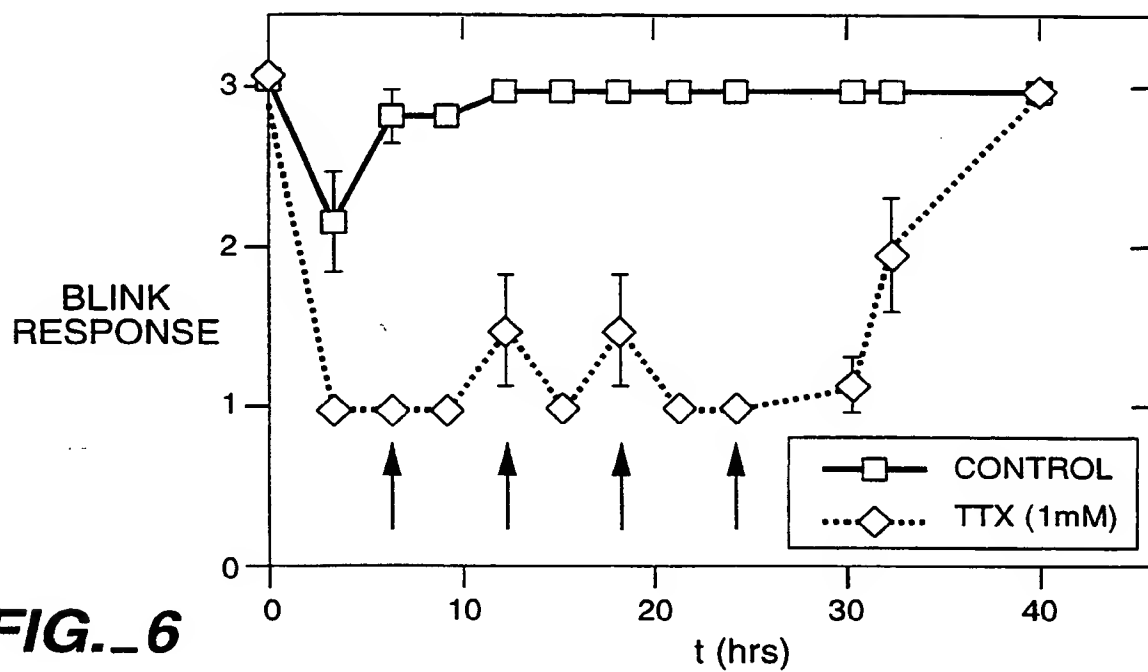
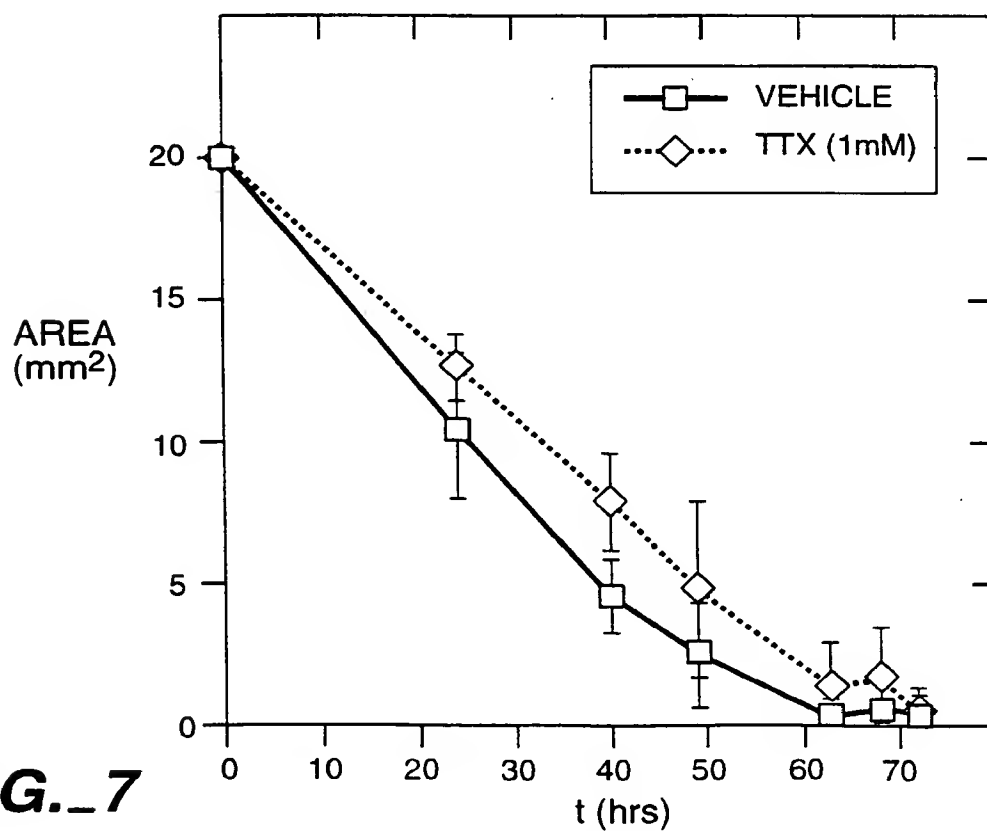
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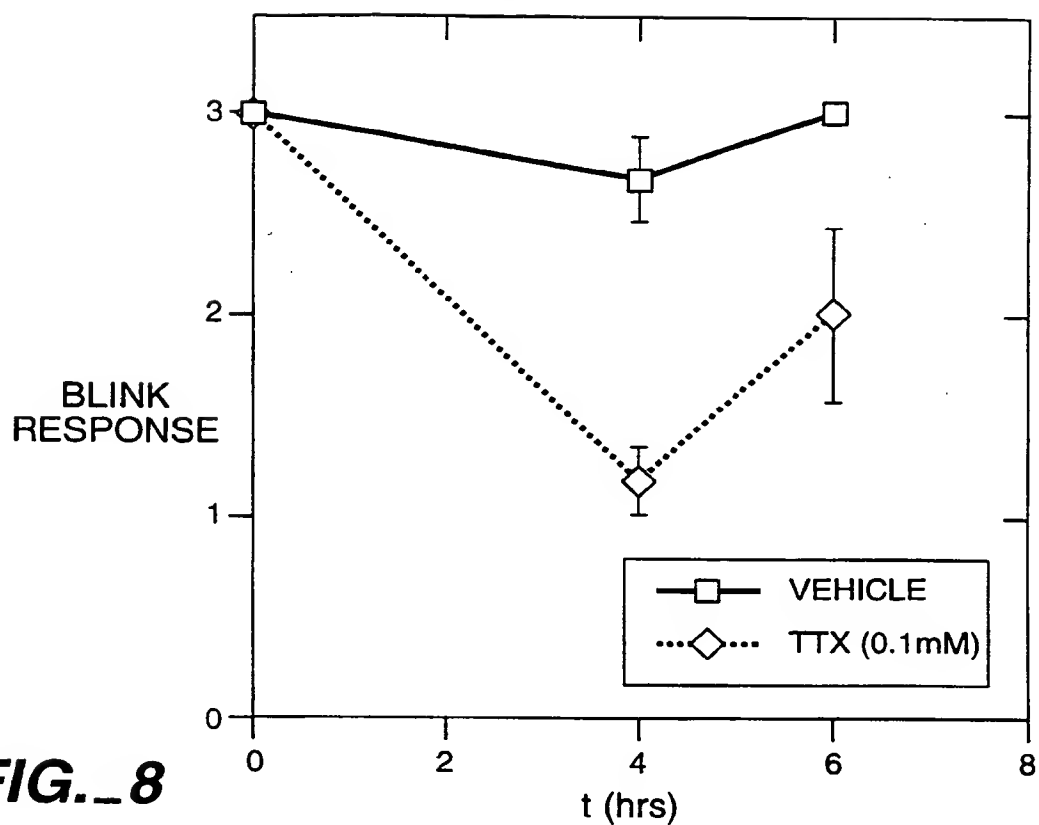
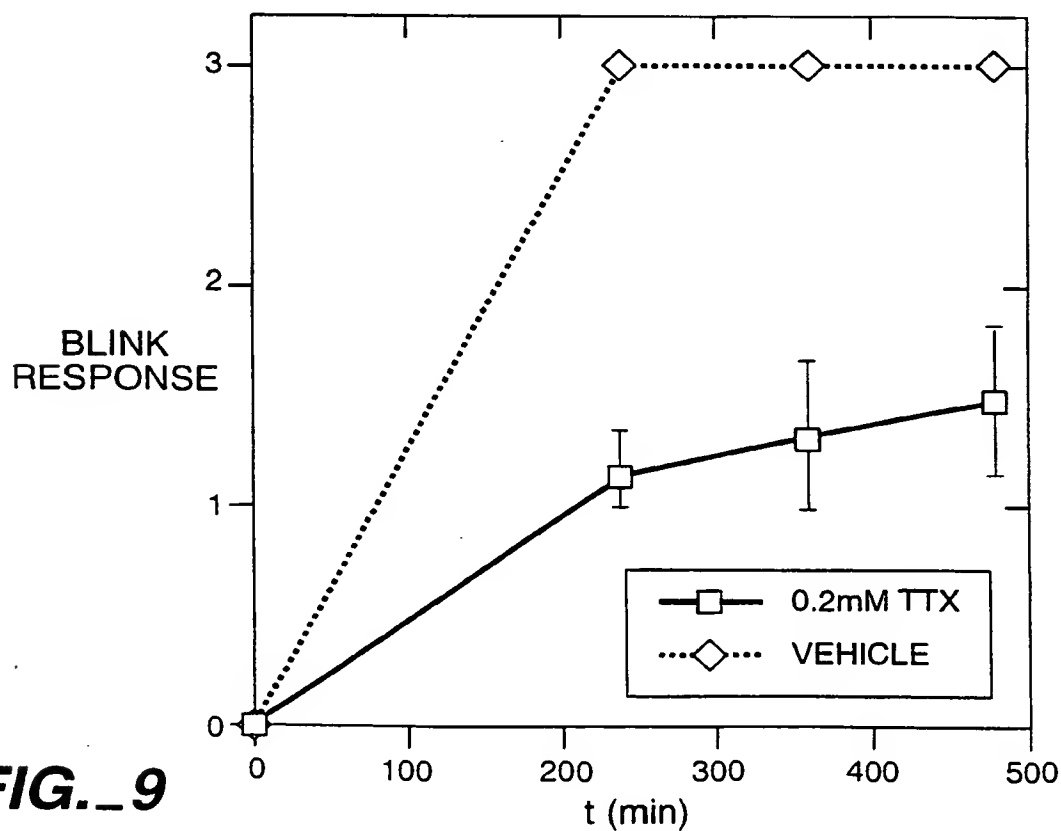
**FIG. 4****FIG. 5**

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**FIG._6****FIG._7**

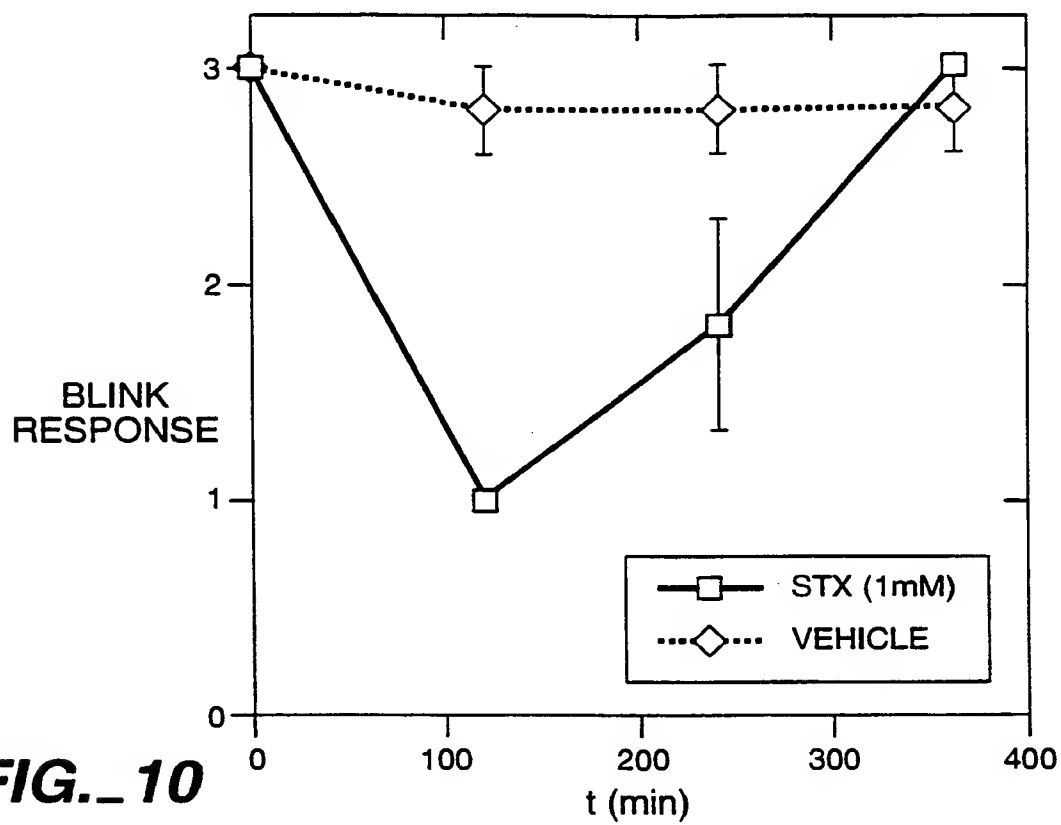
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**FIG. 8****FIG. 9**

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**FIG. 10**



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US98/06705 (22) International Filing Date: 2 April 1998 (02.04.98) (30) Priority Data: 60/040,903 2 April 1997 (02.04.97) US 60/076,317 27 February 1998 (27.02.98) US (71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 300 Lakeside Drive, 22nd floor, Oakland, CA 94612-3550 (US). (72) Inventors: SCHWARTZ, Daniel, M.; 42 Calhoun Terrace, San Francisco, CA 94133 (US). FIELDS, Howard, L.; 755 Euclid Avenue, Berkeley, CA 94708 (US). (74) Agents: NAKAMURA, Jackie, N.; Cooley Godward LLP, 3000 El Camino Real, Five Palo Alto Square, Palo Alto, CA 94306-2155 (US) et al.		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 30 December 1998 (30.12.98)
(54) Title: METHOD OF LOCAL ANESTHESIA (57) Abstract A method of producing local anesthesia in a mammal experiencing pain in an epithelial tissue region is described. The method includes topically administering to the region, in a suitable pharmaceutical vehicle, an effective dose of a long-acting sodium channel blocking compounds, such as tetrodotoxin or saxitobin.		

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/06705

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/505

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHANG, YONGJIAN ET AL: "The local anesthetic activity and toxicity of tetrodotoxin in combination with usual local anesthetics" HEBEI YIXUEYUAN XUEBAO (1989), 10(1), 9-13 CODEN: HYXUEI, XP002075602 see abstract	1-5, 7-12, 15-21, 23,24, 26-30
X	ADAMS, H. J. ET AL: "The local anesthetic activity of saxitoxin alone and with vasoconstrictor and local anesthetic agents" ARCH. INT. PHARMACODYN. THER. (1976), 224(2), 275-82 CODEN: AIPTAK, XP002075603 see abstract see page 278, paragraph 3 - page 280, paragraph 3; tables 1-4 --- -/-	1-4,6,8, 9,12-18, 23,24, 26,31



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the international search

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

A. Jakobs

INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OGURA Y ET AL: "Mechanism of local anesthetic action of crystalline tetrodotoxin and its derivatives." EUROPEAN JOURNAL OF PHARMACOLOGY, (1968 APR) 3 (1) 58-67. JOURNAL CODE: EN6. ISSN: 0014-2999., XP002075604 Netherlands cited in the application see page 60, column 2, paragraph 3 - page 65, column 1, paragraph 1; tables 1-7 ---	1-5, 7-12, 15-21, 23,27-30
X	YAGIELA J.A.: "Local anesthetics: A century of progress." ANESTH. PROG., (1985) 32/2 (47-56). CODEN: ANPRBG, XP002075605 United States see page 53, column 2, paragraph 4; figure 3 ---	1-5,7, 12,27-30
X	DATABASE WPI Section Ch, Week 8035 Derwent Publications Ltd., London, GB; Class B02, AN 80-61332C XP002075609 & JP 55 094 319 A (ODAKA Y) see abstract ---	1-5,7, 10-12, 27-30
X	BYNKE, GUNNEL ET AL: "Is substance P necessary for corneal nociception?" EUR. J. PHARMACOL. (1984), 101(3-4), 253-8 CODEN: EJPHAZ; ISSN: 0014-2999, XP002075606 see abstract; table 1 ---	1-5, 7-12, 15-21, 23,27-30
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Y	see column 2, line 15 - column 2, line 65; example 5; tables 6,8 ---	16,22
X	US 3 966 934 A (ADAMS, HERBERT J. F. ET AL) 29 June 1976	1,14
Y	see column 1, line 60 - column 5, line 5; examples 1-7; table 6 ---	16,22
X	US 3 892 847 A (ADAMS HERBERT J F ET AL) 1 July 1975 see claims 1-4; tables 1,2 ---	1,14
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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	SCHWARTZ D M ET AL: "Experimental use of tetrodotoxin for corneal pain after excimer laser keratectomy." CORNEA, (1998 MAR) 17 (2) 196-9. JOURNAL CODE: DSN. ISSN: 0277-3740., XP002075607 United States see the whole document ---	1-12, 16-21, 23,25, 27-30
A	UNGER, W. G. ET AL: "Prostaglandin and neurogenically mediated ocular response to laser irradiation of the rabbit iris" EXP. EYE RES. (1977), 25(3), 209-20 CODEN: EXERA6, XP002075608 see the whole document -----	1-31

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(54) Title: METHOD OF LOCAL ANESTHESIA (57) Abstract <p>A method of producing local anesthesia in a mammal experiencing pain in an epithelial tissue region is described. The method includes topically administering to the region, in a suitable pharmaceutical vehicle, an effective dose of a long-acting sodium channel blocking compounds, such as tetrodotoxin or saxitobin.</p>		

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AMENDED CLAIMS

[received by the International Bureau on 2 December 1998 (02.12.98);
original claims 15 and 17 cancelled; original claims 1, 16, 23, 25, 27, 28, 29, 31 and 32 amended;
new claims 33-40 added; remaining claims unchanged (5 pages)]

1. A method of producing local anesthesia in a partially or completely de-
5 epithelialized tissue region of a mammal, comprising
topically administering an effective dose of a long-acting sodium channel
blocking compound, in a pharmaceutically suitable vehicle, to said de-epithelialized
tissue region of said mammal, wherein said de-epithelialized tissue region is a corneal
region, a region in the upper or lower gastrointestinal tract, or a genital lesion in the
10 genital area.

2. The method of claim 1, wherein said long-acting sodium channel
blocking compound does not inhibit re-epithelialization of said epithelial tissue
region.

15 3. The method of claim 2, wherein said long-acting sodium channel
blocking compound is a compound capable of specifically binding to a site on an
extracellular region of a sodium channel alpha subunit.

20 4. The method of claim 3, wherein said site is on an SS2 extracellular
region of a sodium channel alpha subunit.

5. The method of claim 4, wherein said long-acting sodium channel
blocking compound is tetrodotoxin.

25 6. The method of claim 4, wherein said long-acting sodium channel
blocking compound is saxitoxin.

7. The method of claim 5, wherein said effective dose of tetrodotoxin is
30 administered from a formulation containing tetrodotoxin at a concentration of
between 0.001-10 mM.

8. The method of claim 1, wherein said administering is to a partially or completely de-epithelialized corneal tissue region.

5 9. The method of claim 1, wherein said administering includes instillation of drops of said sodium channel blocking compound to the eye following corneal surgery.

10 10. The method of claim 5, wherein said tetrodotoxin is administered in a vehicle having a pH of between 4-8.

11. The method of claim 10, wherein said vehicle has a pH of between 5-7.5.

12. The method of claim 2, wherein said sodium channel blocking compound is administered every 6-8 hours for between about 24-72 hours.

13. The method of claim 1, wherein said de-epithelialized tissue region is a region in the upper or lower gastrointestinal tract.

14. The method of claim 1, wherein said epithelial tissue region is associated with genital lesions in the genital area.

16. A method of producing local anesthesia in an eye of a mammal, comprising
25 topically administering to the corneal surface of the eye of said mammal, in an ophthalmically suitable vehicle, an ophthalmically effective dose of a long-acting sodium channel blocking compound, said corneal surface having an epithelial layer that is partially or completely de-epithelialized.

18. The method of claim 16, wherein said long-acting sodium channel blocking compound is a compound capable of specifically binding to a site on an extracellular region of a sodium channel alpha subunit, wherein said site is on either an SS1 region or an SS2 region.

19. The method of claim 18, wherein said long-acting sodium channel blocking compound is tetrodotoxin and said effective dose is administered from a formulation containing tetrodotoxin at a concentration between about 0.001-10 mM.

5

20. The method of claim 19, wherein said long-acting sodium channel blocking compound is tetrodotoxin and said effective dose is administered from a formulation containing tetrodotoxin at a concentration between about 0.01 mM to 0.2 mM.

10

21. The method of claim 20, wherein said tetrodotoxin is administered in a vehicle having a pH of between about 4-8.

22. The method of claim 16, further comprising
15 topically administering an ophthalmically effective dose of proparacaine to the corneal surface of the eye of said mammal, in an ophthalmically suitable vehicle, before topically administering said long-acting sodium channel blocking compound.

23. A method of reducing pain in a mammal following corneal refractive
20 surgery, comprising,

topically administering to a partially or completely de-epithelialized corneal surface of an eye of said mammal, in an ophthalmically suitable vehicle, an ophthalmically effective dose of a long-acting sodium channel blocking compound.

24. The method of Claim 23, further comprising the step of instilling drops
25 of a non-steroidal anti-inflammatory compound in the eye of said mammal.

25. A method of reducing pain in a mammal following corneal refractive surgery, comprising,
30 topically administering to a corneal surface of an eye of said mammal, in an ophthalmically suitable vehicle, an ophthalmically effective dose of a long-acting sodium channel blocking compound, wherein said administering is by applying to the

eye of said mammal a bandage contact lens, wherein said lens is capable of delivering said long-acting sodium channel blocking compound to said corneal surface.

26. The method of claim 23, further comprising administering to said
5 mammal an antibiotic or a non-steroidal anti-inflammatory drug.

27. As an article of manufacture, an ophthalmically acceptable dosage container comprising:

(a) from about 0.003 μg to 160 μg of tetrodotoxin in an ophthalmically
10 acceptable vehicle at pH of between about 4-8.

28. The article of claim 27, wherein said container comprises from about 0.127 μg to 2.54 μg of tetrodotoxin.

29. A local anesthetic composition which comprises an ophthalmically
15 effective amount of tetrodotoxin, in a concentration of 0.01 mM to 1 mM, in an ophthalmically acceptable vehicle at pH 4-6.

30. The composition of claim 29, wherein said concentration is between
20 0.01 mM and 0.2 mM tetrodotoxin.

31. The composition of claim 29, wherein said concentration is between 0.01 mM and 0.1 mM tetrodotoxin.

32. A local anesthetic composition which comprises an ophthalmically
25 effective amount of saxitoxin, in a concentration of 0.1 mM to 10 mM, in an ophthalmically acceptable vehicle at pH 4-8.

33. A local anesthetic composition which comprises an ophthalmically
30 effective amount of tetrodotoxin, in a concentration of 0.1 mM to 10 mM, in an ophthalmically acceptable vehicle at pH 4-8.

34. The composition of claim 33, wherein said concentration is between 0.1 mM and 0.2 mM tetrodotoxin.

5 35. Use of a long-acting sodium channel blocking compound for the manufacture of a medicament for use in producing local anesthesia in a partially or completely de-epithelialized corneal, gastrointestinal, or genual tissue region.

36. The use according to claim 35, wherein said sodium channel blocking compound is tetrodotoxin.

10

37. The use according to claim 35, wherein said sodium channel blocking compound is saxitoxin.

15 38. Use of a long-acting sodium channel blocking compound for the manufacture of a medicament for use in producing local anesthesia in a partially or completely de-epithelialized tissue region in an eye of a mammal.

20

39. The use according to claim 38, wherein said sodium channel blocking compound is tetrodotoxin.

25

40. Use of a long-acting sodium channel blocking compound for the manufacture of a medicament for use in reducing pain in a de-epithelialized corneal surface in an eye of a mammal following corneal refractive surgery.

STATEMENT UNDER ARTICLE 19

The International Search Report identified ZHANG, Hebei Yixueyuan Xuebao (1989) 10(1), 9-13, Abstract XP002075602, ADAMS, Arch. Int. Pharmacodyn. Ther. (1976) 224(2), 275-82, Abstract XP002075603, OGURA, European Journal of Pharmacology 3 (1968), pp. 58-67, North-Holland Publ. Comp., Amsterdam, YAGIELA, Anesth. Prog. (1985) 32/2 (47-56), Abstract No. XP002075605, Japanese Patent No. 55-04319 filing date 7/17/70, BYNKE, European Journal of Pharmacology, Vol. 101, pp. 253-258, 1984, U.S. Patent 3,892,847 as Category X references; German Patent DE 21 63 055 A filed 12.10.72, U.S. Patent 4,022,899, and U.S. Patent 3,966,934 as Category X references.

Adams et al. discloses the application of 1-10 ug/ml saxitoxin and tetrodotoxin to an intact cornea. However, Adams et al. does not teach the use of saxitoxin or tetrodotoxin in de-epithelialized tissue, as set forth in the specification on page 2, lines 17-19, page 9, lines 21-31, page 10, lines 3-6, and page 13-14, and recited in amended Claims 1-14, 16, 18-24 and 26, and new Claims 35-40. Nor does Adams et al. teach a composition comprising as much as 0.1 mM saxitoxin or tetrodotoxin, as described in the specification on page 13 and recited in new Claims 33-34.

Ogura et al. discloses the application of 0.03 mM (or less) tetrodotoxin, pH 6.65, to the intact cornea. However, Ogura et al. does not teach the use of tetrodotoxin in de-epithelialized tissue. Nor does Ogura et al. teach a composition comprising as much as 0.1 mM saxitoxin or tetrodotoxin. Nor does Ogura et al. teach a composition having a pH less than 6.65, as set forth in the specification on page 4, lines 4-5, page 5, lines 12-17, and in the Examples, and recited in amended Claims 29-31.

Yagiela et al. teaches against the use of tetrodotoxin as an anesthetic because of systemic toxicity and poor lipid solubility. However, lipid solubility is not applicable to an abraded corneal epithelium where the lipid barrier has been removed.

Bynke et al. discloses that intravitreal injection of tetrodotoxin abolishes corneal sensitivity for 2-4 hours. However, the effects of tetrodotoxin on de-epithelialized cornea is not taught.

Unger et al. discloses topical application of 2 and 10 μg of tetrodotoxin in rabbits but does not teach the use of tetrodotoxin on de-epithelialized tissue.

The '899, '934, and '847 patents discloses an injectable anesthetic combination of tetrodotoxin and a conventional anesthetic compound. However, a topical combination composition is not taught.

Thus, Adams et al., Ogura et al., Yagiela et al., Bynke et al., Unger et al., and the '899, '934, and '847 patents do not disclose the methods and compositions recited in the amended and new claims.